Glyphosate

Annexure — XIV

p. 01 to 751

SYNOPSIS

- Introduction
- Discovery of glyphosate
- Impurities
- Formulations
- Mode of action
- Uses
- Residues in food products
- Toxicity
- Acute toxicity
- Human
- Toxicology Of "Inert" Ingredients In Glyphosate Containing Products
- Carcinogenicity
- Glyphosate Opinion And Transparency
- Genotoxicity
- Genetic damage
- Developmental or Reproductive Toxicity
- Neurotoxicity
- Endocrine Disruption
- Environmental Fate
- Eco-Toxicity
    a) Aquatic Fauna
    b) Toxic to Fish
    c) Toxic invertebrates
    d) Monarch butterfly populations
    e) Antimicrobial activity
    f) Micro-organisms
    g) Soil biota
    h) Toxicity to Birds
    i) Honey Bees
    j) Earthworms
    k) Effect on plant health
    l) Genetically modified crops
- Government And Organization Positions
- European Food Safety Authority
- US Environmental Protection Agency
- International Agency for Research on Cancer
- California Office of Environmental Health Hazard Assessment
- European Chemicals Agency
- European Union
- Other countries
- Lawsuits claiming liability for cancer
- Case Study
- Where is Glyphosate Banned?
- Why is Glyphosate Banned?
- Is Glyphosate Banned in Europe?
- Is Glyphosate Banned in the United States?
- References

GLYPHOSATE

1.0 INTRODUCTION

Glyphosate (IUPAC name: ***N*-(phosphonomethyl)glycine**) is a broad-spectrum systemic herbicide and crop desiccant. It is an organophosphorus compound, specifically a phosphonate, which acts by inhibiting the plant enzyme 5-enolpyruvylshikimate-3-phosphate synthase. It is used to kill weeds, especially annual broadleaf weeds and grasses that compete with crops. It was discovered to be an herbicide by Monsanto chemist John E. Franz in 1970. Monsanto brought it to market for agricultural use in 1974 under the trade name Roundup. Monsanto's last commercially relevant United States patent expired in 2000. Farmers quickly adopted glyphosate for agricultural weed control, especially after Monsanto introduced glyphosate-resistant Roundup Ready crops, enabling farmers to kill weeds without killing their crops. In 2007, glyphosate was the most used herbicide in the United States' agricultural sector and the second-most used (after 2,4-D) in home and garden, government and industry, and commercial applications (United States EPA 2007).

Glyphosate is absorbed through foliage, and minimally through roots, and transported to growing points. It inhibits a plant enzyme involved in the synthesis of three aromatic amino acids: tyrosine, tryptophan, and phenylalanine. It is therefore effective only on actively growing plants and is not effective as a pre-emergence herbicide. An increasing number of crops have been genetically engineered to be tolerant of glyphosate (e.g. Roundup Ready soybean, the first Roundup Ready crop, also created by Monsanto), which allows farmers to use glyphosate as a post-emergence herbicide against weeds.

1.1. DISCOVERY OF GLYPHOSATE

Glyphosate was first synthesized in 1950 by Swiss chemist Henry Martin, who worked for the Swiss company Cilag. The work was never published (Dill, *et al.*, 2010). Somewhat later, glyphosate was independently discovered in the United States at Monsanto in 1970. Monsanto chemists had synthesized about 100 derivatives of amino methylphosphonic acid as potential water-softening agents. Two were found to have weak herbicidal activity, and John E. Franz, a chemist at Monsanto, was asked to try to make analogs with stronger herbicidal activity. Monsanto developed and patented the use of glyphosate to kill weeds in the early 1970s and first brought it to market in 1974, under the

Roundup brandname (Duke and Powles, 2008). While its initial patent expired in 1991, Monsanto retained exclusive rights in the United States until its patent on the isopropylamine salt expired in September 2000 (Fernandez, 2002).

In 2008, United States Department of Agriculture (USDA) Agricultural Research Service (ARS) scientist Stephen O. Duke and Stephen B. Powles—an Australian weed expert—described glyphosate as a "virtually ideal" herbicide (Duke and Powles, 2008). In 2010 Powles stated: "glyphosate is a one in a 100-year discovery that is as important for reliable global food production as penicillin is for battling disease (Powles, 2010). As of April 2017, the Canadian government stated that glyphosate was "the most widely used herbicide in Canada", at which date the product labels were revised to ensure a limit of 20% POEA by weight.

1.2 IMPURITIES

Technical grade glyphosate is a white powder which, according to FAO specification, should contain not less than 95% glyphosate. Formaldehyde, classified as a known human carcinogen, (International Agency for Research on Cancer, 2006) and *N*-nitrosoglyphosate, have been identified as toxicologically relevant impurities (FAO, 2014). The FAO specification limits the formaldehyde concentration to a maximum of 1.3 g/kg glyphosate. *N*-Nitrosoglyphosate, "belonging to a group of impurities of particular concern as they can be activated to genotoxic carcinogens (European Food Safety Authority, 2015). should not exceed 1 ppm. (FAO, 2014).

1.3. Formulations

Glyphosate is marketed in the United States and worldwide by many agrochemical companies, in different solution strengths and with various adjuvants, under dozens of tradenames (Tu et al., 2001). As of 2010, more than 750 glyphosate products were on the market. In 2012, in terms of volume about half of the total global consumption of glyphosate was for agricultural crops; the forestry sector is another important market. Asia and the Pacific was the largest and fastest growing regional market. Chinese manufacturers collectively are the world's largest producers of glyphosate and its precursors and account for about 30% of global exports. Key manufacturers include Anhui Huaxing Chemical Industry Company, BASF, Bayer CropScience (which also acquired the maker of glyphosate, Monsanto), Dow AgroSciences, DuPont, Jiangsu Good Harvest-Weien Agrochemical Company, Nantong Jiangshan Agrochemical & Chemicals Co., Nufarm,

SinoHarvest, Syngenta, and Zhejiang Xinan Chemical Industrial Group Company (Monsanto, 2011 and China Research & Intelligence, 2013.

Glyphosate is an acid molecule, so it is formulated as a salt for packaging and handling. Various salt formulations include isopropylamine, diammonium, monoammonium, or potassium as the counterion. The active ingredient of the Monsanto herbicides is the isopropylamine salt of glyphosate. Another important ingredient in some formulations is the surfactant polyethoxylated tallow amine. Some brands include more than one salt. Some companies report their product as acid equivalent (ae) of glyphosate acid, or some report it as active ingredient (ai) of glyphosate plus the salt, and others report both. To compare performance of different formulations, knowledge of how the products were formulated is needed. Given that different salts have different weights, the acid equivalent is a more accurate method of expressing and comparing concentrations. Adjuvant loading refers to the amount of adjuvant already added to the glyphosate product (Tu and Randall, 2003 ; Curran et al., 1999).

2.0 MODE OF ACTION

Glyphosate interferes with the shikimate pathway, which produces the aromatic amino acids phenylalanine, tyrosine and tryptophan in plants and microorganisms – but does not exist in the genome of mammals, including humans. It blocks this pathway by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), which catalyzes the reaction of shikimate-3-phosphate (S3P) and phosphoenolpyruvate to form 5-enolpyruvyl-shikimate-3-phosphate (EPSP). Glyphosate is absorbed through foliage and minimally through roots, meaning that it is only effective on actively growing plants and cannot prevent seeds from germinating (Steinrücken and Amrhein, 1980). After application, glyphosate is readily transported around the plant to growing roots and leaves and this systemic activity is important for its effectiveness (Glyphosate technical fact sheet, 2015). Inhibiting the enzyme causes shikimate to accumulate in plant tissues and diverts energy and resources away from other processes, eventually killing the plant. While growth stops within hours of application, it takes several days for the leaves to begin turning yellow (Hock and Elstner, 2004).

3.0. USES

Estimated use of glyphosate in the US in 2013 and estimated total use from 1992–2013. Glyphosate is effective in killing a wide variety of plants, including grasses and broad leaf and woody plants. By volume, it is one of the most widely used herbicides. In 2007, glyphosate was the most used herbicide in the United States agricultural sector, with 180 to 185 million pounds (82,000 to 84,000 tonnes) applied, the second-most used in home and garden with 5 to 8 million pounds (2,300 to 3,600 tonnes) and government applied 13 to 15 million pounds (5,900 to 6,800 tonnes) in industry and commerce (Glyphosate technical fact sheet, 2015). It is commonly used for agriculture, horticulture, viticulture, and silviculture purposes, as well as garden maintenance (including home use). It has a relatively small effect on some clover species and morning glory (Knezevic, 2010). Glyphosate and related herbicides are often used in invasive species eradication and habitat restoration, especially to enhance native plant establishment in prairie ecosystems. The controlled application is usually combined with a selective herbicide and traditional methods of weed eradication such as mulching to achieve an optimal effect (Nyamai, et al., 2011).

Glyphosate is also used for crop desiccation (siccation) to increase harvest yield and uniformity. Glyphosate itself is not a chemical desiccant; rather glyphosate application just before harvest kills the crop plants so that the food crop dries from environmental conditions ("dry-down") more quickly and evenly. Because glyphosate is systemic, excess residue levels can persist in plants due to incorrect application and this may render the crop unfit for sale. When applied appropriately, it can promote useful effects. In sugarcane, for example, glyphosate application increases sucrose concentration before harvest. In grain crops (wheat, barley, oats), uniformly dried crops do not have to be windrowed (swathed and dried) prior to harvest, but can easily be straight-cut and harvested. This saves the farmer time and money, which is important in northern regions where the growing season is short, and it enhances grain storage when the grain has a lower and more uniform moisture content (Gravois and Kenneth 2017 ; Fowler, 2017).

4.0. RESIDUES IN FOOD PRODUCTS

According to the National Pesticide Information Center fact sheet, glyphosate is not included in compounds tested for by the Food and Drug Administration's Pesticide Residue Monitoring Program, nor in the United States Department of Agriculture's Pesticide Data Program. However, a field test showed that lettuce, carrots, and barley contained glyphosate

residues up to one year after the soil was treated with 3.71 lb of glyphosate per acre (4.15 kg per hectare). The U.S. has determined the acceptable daily intake of glyphosate at 1.75 milligrams per kilogram of bodyweight per day (mg/kg/bw/day) while the European Union has set it at 0.5 (European Commission, 2017).

Pesticide residue controls carried out by EU Member States in 2016 analysed 6,761 samples of food products for glyphosate residues. 3.6% of the samples contained quantifiable glyphosate residue levels with 19 samples (0.28%) exceeding the European maximum residue levels (MRLs), which included six samples of honey and other apicultural products (MRL = 0.05 mg/kg) and eleven samples of buckwheat and other pseudo-cereals (MRL = 0.1 mg/kg). Glyphosate residues below the European MRLs were most frequently found in dry lentils, linseeds, soya beans, dry peas, tea, buckwheat, barley, wheat and rye. European Food Safety Authority (July 2018).

TOXICITY

Acute toxicity

Amongst mammals, glyphosate is considered to have "low to very low toxicity". The LD_{50} of glyphosate is 5,000 mg/kg for rats, 10,000 mg/kg in mice and 3,530 mg/kg in goats. The acute dermal LD_{50} in rabbits is greater than 2,000 mg/kg. Indications of glyphosate toxicity in animals typically appear within 30 to 120 minutes following ingestion of a large enough dose, and include initial excitability and tachycardia, ataxia, depression, and bradycardia, although severe toxicity can develop into collapse and convulsions.

A review of unpublished short-term rabbit-feeding studies reported severe toxicity effects at 150 mg/kg/day and "no observed adverse effect level" doses ranging from 50 to 200 mg/kg/day. Glyphosate can have carcinogenic effects in nonhuman mammals. These include the induction of positive trends in the incidence of renal tubule carcinoma and haemangiosarcoma in male mice, and increased pancreatic islet-cell adenoma in male rats. In reproductive toxicity studies performed in rats and rabbits, no adverse maternal or offspring effects were seen at doses below 175–293 mg/kg of body weight per day [Kimmel *et al* 2013]. Glyphosate-based herbicides may cause life-threatening arrhythmias in mammals. Evidence also shows that such herbicides cause direct electrophysiological changes in the cardiovascular systems of rats and rabbits [Gress et al., 2015]

Human

The acute oral toxicity for mammals is low, but death has been reported after deliberate overdose of concentrated formulations. The surfactants in glyphosate formulations can increase the relative acute toxicity of the formulation In a 2017 risk assessment, the European Chemicals Agency (ECHA) wrote: "There is very limited information on skin irritation in humans. Where skin irritation has been reported, it is unclear whether it is related to glyphosate or co-formulants in glyphosate-containing herbicide formulations." The ECHA concluded that available human data was insufficient to support classification for skin corrosion or irritation. Inhalation is a minor route of exposure, but spray mist may cause oral or nasal discomfort, an unpleasant taste in the mouth, or tingling and irritation in the throat. Eye exposure may lead to mild conjunctivitis. Superficial corneal injury is possible if irrigation is delayed or inadequate. (Van Bruggen, et al., 2018 ; Sribanditmongkol, et al., 2012 and Bradberry, 2004).

Acute toxicity and chronic toxicity are dose-related. Skin exposure to ready-to-use concentrated glyphosate formulations can cause irritation, and photocontact dermatitis has been occasionally reported. These effects are probably due to the preservative benzisothiazolin-3-one. Severe skin burns are very rare. Inhalation is a minor route of exposure, but spray mist may cause oral or nasal discomfort, an unpleasant taste in the mouth, or tingling and irritation in the throat. Eye exposure may lead to mild conjunctivitis. Superficial corneal injury is possible if irrigation is delayed or inadequate. Death has been reported after deliberate overdose. Ingestion of Roundup ranging from 85 to 200 ml (of 41% solution) has resulted in death within hours of ingestion, although it has also been ingested in quantities as large as 500 ml with only mild or moderate symptoms. Adult consumption of more than 85 ml of concentrated product can lead to corrosive esophageal burns and kidney or liver damage. More severe cases cause "respiratory distress, impaired consciousness, pulmonary edema, infiltration on chest X-ray, shock, arrhythmias, renal failure requiring haemodialysis, metabolic acidosis, and hyperkalaemia" and death is often preceded by bradycardia and ventricular arrhythmias. While the surfactants in formulations generally do not increase the toxicity of glyphosate itself, it is likely that they contribute to its acute toxicity. [Nguyen, et al., 2016].

TOXICOLOGY OF "INERT" INGREDIENTS IN GLYPHOSATE CONTAINING PRODUCTS

- Three glyphosate products contain **ammonium sulfate**. It causes eye irritation, nausea and diarrhea, and may cause allergic respiratory reactions. Prolonged exposure can cause permanent eye damage.
- One glyphosate product contains **benzisothiazolone**. It causes eczema, skin irritation, and a light-induced allergic reaction in sensitive people.
- Four glyphosate products contain **3-iodo-2-propynyl butylcarbamate (IPBC).** It is severely irritating to eyes and increases the incidence of miscarriages in laboratory tests.51 It also can cause allergic skin reactions.
- One glyphosate product contains **isobutane.** It causes nausea, nervous system depression, and difficulty breathing. It is a severe fire hazard.
- One glyphosate product contains **methyl pyrrolidinone**. It causes severe eye irritation. It has caused fetal loss and reduced fetal weights in laboratory animals.
- Three glyphosate products contain **pelargonic acid**. It causes severe eye and skin irritation and may cause respiratory tract irritation.
- Nine glyphosate products contain **polyethoxylated tallowamine (POEA).** It causes eye burns; skin redness, swelling, and blistering; nausea; and diarrhea.
- Three glyphosate products contain **potassium hydroxide**. It causes irreversible eye injury, deep skin ulcers, severe digestive tract burns, and severe irritation of the respiratory tract.
- One glyphosate product contains sodium **sulfite**. It may cause eye and skin irritation with vomiting and diarrhea as well as skin allergies. Exposure to small amounts can cause severe allergic reactions.
- Three glyphosate products contain **sorbic acid**. It may cause severe skin irritation, nausea, vomiting, chemical pneumonitis, and sore throat. It also causes allergic reactions.
- **Isopropylamine** is used in some Roundup products. It is "extremely destructive to tissue of the mucous membranes and upper respiratory tract." Symptoms of exposure are wheezing, laryngitis, headache, and nausea.

CARCINOGENICITY

In March 2015, the World Health Organization's International Agency for Research on Cancer (IARC) classified glyphosate as "probably carcinogenic in humans" (category 2A) based on epidemiological studies, animal studies, and *in vitro* studies. In contrast, the European Food Safety Authority concluded in November 2015 that "the substance is unlikely to be genotoxic (i.e. damaging to DNA) or to pose a carcinogenic threat to humans", later clarifying that while carcinogenic glyphosate-containing formulations may exist, studies "that look solely at the active substance glyphosate do not show this effect." The WHO and FAO Joint committee on pesticide residues issued a report in 2016 stating the use of glyphosate formulations does not necessarily constitute a health risk, and giving admissible daily maximum intake limits (one milligram/kg of body weight per day) for chronic toxicity. The European Chemicals Agency (ECHA) classified glyphosate as causing serious eye damage and toxic to aquatic life, but did not find evidence implicating it as a carcinogen, a mutagen, toxic to reproduction, nor toxic to specific organs (Cressey, 2015 ; Schinasi and Leon, 2014 ; Guyton et al, 2015 ; *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 2016)* .

The German Federal Institute for Risk Assessment toxicology review in 2013 found that "the available data is contradictory and far from being convincing" with regard to correlations between exposure to glyphosate formulations and risk of various cancers, including non-Hodgkin lymphoma (NHL). A meta-analysis published in 2014 identified an increased risk of NHL in workers exposed to glyphosate formulations (Renewal Assessment Report: Glyphosate, 2013).

The consensus among national pesticide regulatory agencies and scientific organizations is that labeled uses of glyphosate have demonstrated no evidence of human carcinogenicity. The Joint FAO/WHO Meeting on Pesticide Residues (JMPR), the European Commission, the Canadian Pest Management Regulatory Agency, the Australian Pesticides and Veterinary Medicines Authority and the German Federal Institute for Risk Assessment have concluded that there is no evidence that glyphosate poses a carcinogenic or genotoxic risk to humans. The EPA has classified glyphosate as "not likely to be carcinogenic to humans."[100][101] One international scientific organization, the International Agency for Research on Cancer, classified glyphosate in Group 2A, "probably carcinogenic to humans" in 2015.

There is weak evidence human cancer risk might increase as a result of occupational exposure to large amounts of glyphosate, such as agricultural work, but no good evidence of such a risk from home use, such as in domestic gardening. According to a systematic review and meta-analysis published in 2016, when weak statistical associations with cancer have been found, such observations have been attributed to bias and confounding in correlational studies due to workers often being exposed to other known carcinogens Cancer Research UK. 2016.

A meta-analysis published in 2019 looked at whether there was an association between an increased risk of non-Hodgkin lymphoma in humans and high cumulative exposures to glyphosate-based herbicides. The analysis used the most recent update of the Agricultural Health Study cohort published in 2018 and five case-control studies published in 2019. The research found a "compelling link" between exposures to glyphosate-based herbicides and increased risk for non-Hodgkin lymphoma (Kimmel et al., 2013).

In fact, in 2015 the IARC (International Agency for Research on Cancer, Lyon, France), an organization referred to as the specialized cancer agency of the World Health Organization (WHO, Geneva, Switzerland), classified the substance as "likely carcinogenic" to humans. This triggered an immediate and negative reaction from the producer, who accused the Agency and claimed that they had failed to carry out their studies properly and that these conclusions were largely contradictory to published research. Additionally, in 2015, just a few months after the IARC monography published on glyphosate, the EFSA (European Food Safety Authority, Parma, Italy), another WHO related organization, declared that it was "unlikely" that the molecule could be carcinogenic to humans or that it could cause any type of risk to human health. The conflict between the two organizations of the World Health Organization triggered many doubts, and for this reason, a series of independent studies were launched to better understand what glyphosate's danger to humans and the environment really was. The results have brought to light how massive use of the herbicide has created over time a real global contamination that has not only affected the soil, surface and groundwater as well as the atmosphere, but even food and commonly used objects, such as diapers, medical gauze, and absorbent for female intimate hygiene. How human health is compromised as a result of glyphosate exposure is a topic that is still very debatable and still unclear and unambiguous. **[Sustainability 2018, 10, 950].**

Glyphosate is a broad-spectrum systemic herbicide. Several epidemiological studies on cancer outcomes following occupational exposure to glyphosate were available. The evaluation of these studies focused on the occurrence of NHL. Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta analysis. However, it is notable that the only large cohort study of high quality found no evidence of an association at any exposure level. Glyphosate has been extensively tested for genotoxic effects using a variety of tests in a wide range of organisms. The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg body weight by the oral route, the route most relevant to human dietary exposure, was not associated with genotoxic effects in an overwhelming majority of studies conducted in mammals, a model considered to be appropriate for assessing genotoxic risks to humans. The Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures. Several carcinogenicity studies in mice and rats are available. **The Meeting concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses. In** view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet. The Meeting reaffirmed the group ADI for the sum of glyphosate and its metabolites of 0–1 mg/kg body weight on the basis of effects on the salivary gland. The Meeting concluded that it was not necessary to establish an ARfD for glyphosate or its metabolites in view of its low acute toxicity. [**JOINT FAO/WHO MEETING ON PESTICIDE RESIDUES Geneva, 9–13 May 2016].**

The AHS cohort study found no evidence of a positive association of NHL with glyphosate exposure or an exposure–response relationship (De Roos et al., 2005). Elevated risks were reported in various case–control studies. A significant elevated risk of NHL associated with ever- versus neveruse of glyphosate (OR = 2.1; 95% CI = 1.1–4.0) was reported (De Roos et al., 2003). Ever-use of glyphosate was not associated with risk of NHL in the Cross-Canada Case–control Study of Pesticides and Health (McDuffie et al., 2001), but when analysing days of use per year, there was a significant elevated risk in the highest usage category (OR = 2.12; 95% CI = 1.20–3.73; for > 2 days/year glyphosate use). There was, however, no indication of an exposure–response relationship across exposure usage

categories (McDuffie et al., 2001). In another case–control study, a significant increased risk of NHL associated with ever-use (OR = 2.02; 95% CI = 1.10–3.71) as well as the highest usage category (OR = 2.36; 95% CI = 1.04–5.37; for greater than 10 days/year glyphosate use) was observed, with some suggestion of an exposure–response gradient (Eriksson et al., 2008). Two smaller case–control studies with few exposed cases and limited statistical power reported a nonsignificant elevated risk (Hardell et al., 2002) and no association (Orsi et al., 2009), respectively, for risk of NHL and ever-use of glyphosate. glyphosate (Schinasi & Leon, 2014). The meta-analysis, including the AHS, found a significant 50% excess risk ratio for ever- versus never-use of glyphosate (Schinasi & Leon, 2014).

Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta-analysis. However, it is notable that the AHS (De Roos et al., 2005), which is the only cohort study and is large and of high quality, found no evidence of association at any exposure level. **[GLYPHOSATE 89–296 JMPR 2016].**

GLYPHOSATE OPINION AND TRANSPARENCY

One adopted CLH opinion was on glyphosate, a widely used active substance in herbicides. Based on a thorough assessment of the information included in the proposal made by Germany and submitted via public consultation, RAC recommended not to change the existing harmonised classification for glyphosate. This included the conclusion that current scientific evidence does not support the classification of glyphosate for carcinogenicity.

The RAC's consideration of the dossier was divided into two parts. First, interested parties [Health and Environment Alliance (HEAL), IARC, WHO/FAO JMPR, EFSA, Germany as dossier submitter and the Glyphosate Task Force] were invited to provide their views to the Committee on the scientific studies on glyphosate. Then, at its next meeting, RAC discussed the classification based on all available information. It had access to the original study reports on carcinogenicity and carried out its independent evaluation on that basis, also acknowledging later reviews and opinions. It made its handling of the case open to public scrutiny. The ECHA secretariat answered numerous enquiries, including those from the European Parliament, the press and individuals.[ECHA General Report 2017]. The expert report published in March 2017 once again confirms the existing classification: glyphosate does not cause cancer and does not cause organ damage. According to ECHA's assessment,

glyphosate does not have any mutagenic, reprotoxic or genotoxic properties either. The ECHA assessment is therefore in line with the official conclusions of the German Federal Institute for Risk Assessment (BfR), the European Food Safety Authority (EFSA) and the WHO/FAO Joint Meeting on Pesticide Residues (JMPR). A decision is due this year, based on the ECHA ass essment, as to whether glyphosate will continue to be approved in Europe. http://www.glyphosate.eu/european-chemicals-agency-glyphosate-does-not-cause-cancer

The IARC WG concluded that glyphosate is a 'probable human carcinogen', putting it into IARC category 2A due to sufficient evidence of carcinogenicity in animals, limited evidence of carcinogenicity in humans and strong evidence for two carcinogenic mechanisms.

- The IARC WG found an association between NHL and glyphosate based on the available human evidence.
- The IARC WG found significant carcinogenic effects in laboratory animals for rare kidney tumours and hemangiosarcoma in two mouse studies and benign tumours in two rat studies.
- The IARC WG concluded that there was strong evidence of genotoxicity and oxidative stress for glyphosate, entirely from publicly available research, including findings of DNA damage in the peripheral blood of exposed humans. The RAR concluded5 (Vol. 1, p.160 that 'classification and labelling for carcinogenesis is not warranted' and 'glyphosate is devoid of genotoxic potential'.
- EFSA4 classified the human evidence as 'very limited' and then dismissed any association of glyphosate with cancer without clear explanation or justification.
- Ignoring established guidelines cited in their report, EFSA dismissed evidence of renal tumours in three mouse studies, hemangiosarcoma in two mouse studies and malignant lymphoma in two mouse studies.
- Thus, EFSA incorrectly discarded all findings of glyphosate-induced cancer in animals as chance occurrences.
- EFSA ignored important laboratory and human mechanistic evidence of genotoxicity.
- EFSA confirmed that glyphosate induces oxidative stress but then, having dismissed all other findings of possible carcinogenicity, dismissed this finding on the grounds that oxidative stress alone is not sufficient for carcinogen labelling.[Portier CJ, et al. J Epidemiol Community Health August 2016 Vol 70 No]

California Superior Court ruled that Roundup, which contains glyphosate, was the cause of cancer for Dewayne Johnson, a school groundskeeper. It ordered Monsanto, the manufacturer of the weedicide, to pay $289 (US dollars) million in damages. In India, glyphosate is the most commonly used weedicide.

According to the Directorate Of Plant Protection, Quarantine and Storage, 148 of the 414 metric tonnes of weedicides consumed in the country in 2014-15 was glyphosate. In 2015-16, a little more than 370 metric tonnes of monocrotophos was used on Indian soil. Both these pesticides do not figure in the list in the notification. "It looks like the authorities chose the pesticides, many of which were already on their way out. With delays up to two years, the pesticide industry had enough time to clear their stock," said Kuruganti. [https://www.firstpost.com/india/india-bans-18-pesticides-after-reviewing-66-out-of-104-used-in-country-leaves-out-monocrotophos-glyphosate-4979161.html

A 2000 review concluded that "under present and expected conditions of new use, there is no potential for Roundup herbicide to pose a health risk to humans". A 2012 meta-analysis of epidemiological studies (seven cohort studies and fourteen case-control studies) of exposure to glyphosate formulations found no correlation with any kind of cancer. The 2013 systematic review by the German Institute for Risk Assessment of epidemiological studies of workers who use pesticides, exposed to glyphosate formulations found no significant risk, stating that "the available data are contradictory and far from being convincing". However, a 2014 meta-analysis of the same studies found a correlation between occupational exposure to glyphosate formulations and increased risk of B cell lymphoma, the most common kind of non-Hodgkin lymphoma. Workers exposed to glyphosate were about twice as likely to get B cell lymphoma. A 2016 systematic review and meta-analysis found no causal relationship between glyphosate exposure and risk of any type of lymphohematopoietic cancer including non-Hodgkin lymphoma and multiple myeloma. The same review noted that the positive associations found may be due to bias and confounding. The Natural Resources Defense Council has criticized that review, noting that it was funded by Monsanto [Chang and Delzell, 2016 ; Gary *et al.*, 1997].

A 2015 systematic review of observational studies found that except for an excess of Attention Deficit Hyperactivity Disorder among children born to glyphosate appliers, no evidence that glyphosate exposure among pregnant mothers caused adverse developmental outcomes in their children. Noting the limited size and scope of the review articles available,

the authors noted that "these negative findings cannot be taken as definitive evidence that GLY, at current levels of occupational and environmental exposures, brings no risk for human development and reproduction.

GENOTOXICITY

Genetic damage

Several studies have not found mutagenic effects, so glyphosate has not been listed in the United States Environmental Protection Agency or the International Agency for Research on Cancer databases. Various other studies suggest glyphosate may be mutagenic. The IARC monograph noted that glyphosate-based formulations can cause DNA strand breaks in various taxa of animals *in vitro*.

Glyphosate-containing products are more potent mutagens than glyphosate. The studies include the following:

- In fruit flies, Roundup and Pondmaster (an aquatic herbicide consisting of glyphosate and a trade secret surfactant82) both increased the frequency of sex-linked, recessive lethal mutations. (These are mutations that are usually visible only in males.) Only a single concentration was tested in this study.
- A study of human lymphocytes (a type of white blood cell) showed an increase in the frequency of sister chromatid exchanges following exposure to the lowest dose tested of Roundup.(Sister chromatid exchanges are exchanges of genetic material during cell division between members of a chromosome pair. They result from point mutations.) A 1997 study of human lymphocytes found similar results with Roundup (at both doses tested) and with glyphosate (at all but the lowest dose tested).
- In Salmonella bacteria, Roundup was weakly mutagenic at two concentrations. DNA Adducts (per 10* nucleotides) (averages with standard errors) In onion root cells, Roundup caused an increase in chromosome aberrations, also at two concentrations.
- In mice injected with Roundup, the frequency of DNA adducts (the binding to genetic material of reactive molecules that lead to mutations) in the liver and kidney increased at all three doses tested.
- In another study of mice injected with glyphosate and Roundup, the frequency of chromosome damage and DNA damage increased in bone marrow, liver, and kidney. (Only a single concentration was tested in this study.)

Archives of Toxicology by Koller et al showed increases in nuclear aberrations indicating DNA damage after 20 minutes of exposure to 10 to 20 mg/L of glyphosate. They also found that Roundup was, under all conditions, more active than glyphosate and that there were genotoxic effects after short exposures to a concentration of a 450 dilution of spraying used in agriculture. In conclusion, inhalation could cause DNA damage in exposed agricultural workers.7 Another study looked at why some agricultural workers who use glyphosate have pregnancy problems and showed that it is toxic to human placental JEG-3 cells within 18 hours of exposure in concentrations lower than those in agricultural use—and that this effect increases with concentration and time with Roundup adjuvants. The authors also tested the effects of glyphosate and Roundup at nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. They found that glyphosate disrupts aromatase and mRNA levels and concluded that Roundup, not just glyphosate, has endocrine and toxic effects. [ALTERNATIVE THERAPIES, MAY/JUNE 2014 VOL. 20, 3]

DEVELOPMENTAL OR REPRODUCTIVE TOXICITY

Studies in rats and rabbits indicated that technical glyphosate is not teratogenic. Two multigeneration studies were conducted with technical glyphosate. In the first study, the only effect noted was an increased incidence of unilateral renal tubular dilation in F3b male pups at 30 mg/kg body weight. In the second study, decreased body weights were reported for parents and pups and decreased litter size was associated with dose levels of 30 000 mg/kg diet. Decreased body weights reported for parents and pups at 10 000 mg/kg diet were not toxicologically significant. In parents, the decrease was only 2 to 4% below controls and for pups the decrease was 5.6 to 6.6% lower than controls. The findings in pups were also transient and did not occur consistently in all litters. The NOAEL was 10 000 mg/kg diet. The absence of a renal effect in pups at a higher dose level (1500 mg/kg body weight), though not invalidating earlier findings of unilateral renal tubular dilation in male F3b pups, indicates that the reproducibility of this lesion and its toxicological significance are uncertain. It should be noted that in no other toxicological study was an effect on kidneys found.

NEUROTOXICITY

There was no evidence of neurotoxicity in an acute neurotoxicity study in rats at doses up to 2000 mg/kg bw. The NOAEL for systemic toxicity was 1000 mg/kg bw, based on a single death and general signs of toxicity at 2000 mg/kg bw (Horner, 1996a). In a 90-day

neurotoxicity study in rats. no evidence of neurotoxicity or systemic toxicity was seen at doses up to 20 000 ppm (equal to 1546.5 mg/kg bw per day) (Horner, 1996b).

ENDOCRINE DISRUPTION

In 2007, the EPA selected glyphosate for further screening through its Endocrine Disruptor Screening Program (EDSP). Selection for this program is based on a compound's prevalence of use and does not imply particular suspicion of endocrine activity. On June 29, 2015, the EPA released Weight of Evidence Conclusion of the EDSP Tier 1 screening for glyphosate, recommending that glyphosate not be considered for Tier 2 testing. The Weight of Evidence conclusion stated "...there was no convincing evidence of potential interaction with the estrogen, androgen or thyroid pathways." A review of the evidence by the European Food Safety Authority published in September 2017 showed conclusions similar to those of the EPA report. (United States Environmental Protection Agency, 2015).

Different reports suggest that GBHs may act as endocrine disruptors. Dallegrave et al. (2007) described that exposure to glyphosate-Roundup may induce significant adverse effects on the reproductive system of male Wistar rats at puberty and during adulthood. Gasnier et al. (2009) documented that GBHs are toxic and endocrine disruptors in human cell lines using gene reporter tests. They therefore recommended to consider a real cell impact of glyphosate-based herbicides residues in food, feed or in the environment on human health.

ENVIRONMENTAL FATE

Glyphosate adsorbs strongly to soil, and residues are expected to generally be immobile in soil. Ground and surface water pollution is limited. Glyphosate is readily degraded by soil microbes to aminomethylphosphonic acid (AMPA, which like glyphosate strongly adsorbs to soil solids and is thus unlikely to leach to groundwater). Though both glyphosate and AMPA are commonly detected in water bodies, a portion of the AMPA detected may actually be the result of degradation of detergents rather than from glyphosate (Botta et al., 2009). Glyphosate does have the potential to contaminate surface waters due to its aquatic use patterns and through erosion, as it adsorbs to soil particles suspended in runoff. Detection in surface waters (particularly downstream from agricultural uses) has been reported as both broad and frequent by USGS researchers, although other similar research found equal frequencies of detection in urban-dominated small streams (Battaglin et al., 2014). Rain events can trigger dissolved glyphosate loss in transport-prone soils. The

mechanism of glyphosate sorption to soil is similar to that of phosphate fertilizers, the presence of which can reduce glyphosate sorption. Phosphate fertilizers are subject to release from sediments into water bodies under anaerobic conditions, and similar release can also occur with glyphosate, though significant impact of glyphosate release from sediments has not been established. Limited leaching can occur after high rainfall after application. If glyphosate reaches surface water, it is not broken down readily by water or sunlight (Richards, et al., 2018 ; Muniraa, et al., 2014).

The half-life of glyphosate in soil ranges between 2 and 197 days; a typical field half-life of 47 days has been suggested. Soil and climate conditions affect glyphosate's persistence in soil. The median half-life of glyphosate in water varies from a few to 91 days. At a site in Texas, half-life was as little as three days. A site in Iowa had a half-life of 141.9 days. The glyphosate metabolite AMPA has been found in Swedish forest soils up to two years after a glyphosate application. In this case, the persistence of AMPA was attributed to the soil being frozen for most of the year. Glyphosate adsorption to soil, and later release from soil, varies depending on the kind of soil. Glyphosate is generally less persistent in water than in soil, with 12- to 60-day persistence observed in Canadian ponds, although persistence of over a year has been recorded in the sediments of American ponds. The half-life of glyphosate in water is between 12 days and 10 weeks (Albers et al., 2009 ; Ole and Borggaard, 2011 and Sparling, et al., 2006).

ECO-TOXICITY

Aquatic Fauna

In many freshwater invertebrates, glyphosate has a 48-hour LC_{50} ranging from 55 to 780 ppm. The 96-hour LC_{50} is 281 ppm for grass shrimp (*Palaemonetas vulgaris*) and 934 ppm for fiddler crabs (*Uca pagilator*). These values make glyphosate "slightly toxic to practically non-toxic". Glyphosate products for aquatic use generally do not use surfactants, and aquatic formulations do not use POEA due to aquatic organism toxicity. Due to the presence of POEA, such glyphosate formulations only allowed for terrestrial use are more toxic for amphibians and fish than glyphosate alone. The half-life of POEA (21–42 days) is longer than that for glyphosate (7–14 days) in aquatic environments. Aquatic organism exposure risk to terrestrial formulations with POEA is limited to drift or temporary water pockets where concentrations would be much lower than label rates [Mann et al., 2009].

Some researchers have suggested the toxicity effects of pesticides on amphibians may be different from those of other aquatic fauna because of their lifestyle; amphibians may be more susceptible to the toxic effects of pesticides because they often prefer to breed in shallow, lentic, or ephemeral pools. These habitats do not necessarily constitute formal water-bodies and can contain higher concentrations of pesticide compared to larger water-bodies. Studies in a variety of amphibians have shown the toxicity of GBFs containing POEA to amphibian larvae. These effects include interference with gill morphology and mortality from either the loss of osmotic stability or asphyxiation. At sub-lethal concentrations, exposure to POEA or glyphosate/POEA formulations have been associated with delayed development, accelerated development, reduced size at metamorphosis, developmental malformations of the tail, mouth, eye and head, histological indications of intersex and symptoms of oxidative stress. Glyphosate-based formulations can cause oxidative stress in bullfrog tadpoles.

A 2003 study of various formulations of glyphosate found, "[the] risk assessments based on estimated and measured concentrations of glyphosate that would result from its use for the control of undesirable plants in wetlands and over-water situations showed that the risk to aquatic organisms is negligible or small at application rates less than 4 kg/ha and only slightly greater at application rates of 8 kg/ha.

A 2013 meta-analysis reviewed the available data related to potential impacts of glyphosate-based herbicides on amphibians. According to the authors, the use of glyphosate-based pesticides cannot be considered the major cause of amphibian decline, the bulk of which occurred prior to the widespread use of glyphosate or in pristine tropical areas with minimal glyphosate exposure. The authors recommended further study of species- and development-stage chronic toxicity, of environmental glyphosate levels, and ongoing analysis of data relevant to determining what if any role glyphosate might be playing in worldwide amphibian decline, and suggest including amphibians in standardized test batteries [Ecotoxicological Risk Assessment for Roundup Herbicide", 2019].

Toxic to Fish

Factors important in determining the toxicity of glyphosate or glyphosatecontaining products to fish include the following:

* First, different species of fish have different susceptibilities. For example, coho and chinook salmon are more tolerant of glyphosate than pink or chum salmon.

* Water quality is important: glyphosate in soft water was 20 times more toxic to rainbow trout than was glyphosate in hard water. For Roundup, the reverse is true: it is more toxic in hard water than in soft.

* Age affects the susceptibility of fish because juveniles are often more susceptible than adults. For example, Roundup was four times more toxic to rainbow trout fry and fingerlings than it was to larger fish.

* Nutrition also can determine toxicity. Hungry fish are more susceptible to glyphosate than fed fish. For example, fed flagfish were 10 times more tolerant of glyphosate than unfed fish.

* Finally, glyphosate toxicity increases with increased water temperature. In both rainbow trout and bluegills, toxicity about doubled between 7 and 17^0C (45 and 63^0F). Treatment of riparian areas with glyphosate causes water temperatures to increase for several years following treatment because the herbicide kills shading vegetation. This means that repeated use of glyphosate in a watershed could favor its increased toxicity to fish. In addition, the temperature increase itself could be critical for fish, like juvenile salmon, that are sensitive to water temperature.

Both glyphosate and the commercial products that contain glyphosate are acutely toxic to fish. In general, glyphosate alone is less toxic than the common glyphosate product, Roundup, and other glyphosate products have intermediate toxicity. Part of these differences can be explained by the toxicity of the surfactant (detergent-like ingredient) in Roundup. It is 20 to 70 times more toxic to fish than glyphosate itself.

Toxic invertebrates

It is a very low potential for the compound to build up in the tissues of aquatic invertebrates or other aquatic organisms (EXTOXNET)

Monarch butterfly populations

Use of 2-4 D and other herbicides like glyphosate to clear milkweed along roads and fields may have contributed to a decline in monarch butterfly populations in the Midwestern United States. Along with deforestation and adverse weather conditions, the decrease in milkweed contributed to an 81% decline in monarchs. The Natural Resources Defense Council (NRDC) filed a suit against the EPA in 2015, in which it argued that the agency ignored warnings

about the potentially dangerous impacts of glyphosate usage on monarchs. (Pleasants and Oberhauser , 2013 ; *NRDC Sues EPA Over Demise of Monarch Butterfly Population 2015*)

Antimicrobial activity

The antimicrobial activity of glyphosate has been described in the microbiology literature since its discovery in 1970 and the description of glyphosate's mechanism of action in 1972. Efficacy was described for numerous bacteria and fungi. Glyphosate can control the growth of apicomplexan parasites, such as *Toxoplasma gondii*, *Plasmodium falciparum* (malaria), and *Cryptosporidium parvum*, and has been considered an antimicrobial agent in mammals. Inhibition can occur with some Rhizobium species important for soybean nitrogen fixation, especially under moisture stress [de Araujo et al., 2016].

Micro-organisms

glyphosate may affect bacterial symbionts of animals living near agricultural sites, including pollinators such as bees. The honey bee gut microbiota is dominated by eight bacterial species that promote weight gain and reduce pathogen susceptibility. The gene encoding EPSPS is present in almost all sequenced genomes of bee gut bacteria, indicating that they are potentially susceptible to glyphosate. We demonstrated that the relative and absolute abundances of dominant gut microbiota species are decreased in bees exposed to glyphosate at concentrations documented in the environment. Glyphosate exposure of young workers increased mortality of bees subsequently exposed to the opportunistic pathogen Serratia marcescens. Members of the bee gut microbiota varied in susceptibility to glyphosate, largely corresponding to whether they possessed an EPSPS of class I (sensitive to glyphosate) or class II (insensitive to glyphosate). This basis for differences in sensitivity was confirmed using in vitro experiments in which the EPSPS gene from bee gut bacteria was cloned into Escherichia coli. All strains of the core bee gut species, Snodgrassella alvi, encode a sensitive class I EPSPS, and reduction in S. alvi levels was a consistent experimental result. However, some S. alvi strains appear to possess an alternative mechanism of glyphosate resistance. Thus, exposure of bees to glyphosate can perturb their beneficial gut microbiota, potentially affecting bee health and their effectiveness as pollinators

Soil biota

When glyphosate comes into contact with the soil, it can be bound to soil particles, thereby slowing its degradation. Glyphosate and its degradation product, aminomethylphosphonic acid are considered to be much more benign toxicologically and environmentally than most of the herbicides replaced by glyphosate. A 2016 meta-analysis concluded that at typical application rates glyphosate had no effect on soil microbial biomass or respiration. A 2016 review noted that contrasting effects of glyphosate on earthworms have been found in different experiments with some species unaffected, but others losing weight or avoiding treated soil. Further research is required to determine the impact of glyphosate on earthworms in complex ecosystems. [Rose et al., 2016 ; United States Environmental Protection Agency, 2007]

Toxicity to Birds

Glyphosate is acutely toxic to birds, but only in large amounts. The LC50, the amount in food that kills 50 percent of a population of test animals, is often above 4000 milligrams per kilogram of food Glyphosate also has indirect impacts on birds. Because glyphosate kills plants, its use creates a dramatic change in the structure of the plant community. This affects bird populations, since the birds depend on the plants for food, shelter, and nest support.
For example, a study of four glyphosate-treated clear-cuts (and an unsprayed control plot) in Nova Scotia found that the densities of the two most common species of birds (white-throated sparrow and common yellowthroat) decreased for two years after glyphosate treatment. By the fourth year post-spray, densities had returned to normal for these two species. However, the unsprayed plot had by then been colonized by new species of birds (warblers, vireos, and a hummingbird). These species did not appear on the sprayed plots.

An earlier three year study of songbird abundance following glyphosate treatment of clear-cuts in Maine forests showed similar results. Abundance's of the total number of birds and three common species decreased. The decrease in bird abundance was correlated with decrease in the diversity of the habitat.**(Journal of Pesticide Reform Volume 15, Number 4, Winter 1995)**

Based on current data, EPA has determined that the effects of glyphosate on birds, mammals, fish and invertebrates are minimal. Under certain use conditions, glyphosate may cause adverse effects to non target aquatic plants. Additional data are needed to fully evaluate the effects of glyphosate on nontarget terrestrial plants. Risk reduction measures will be developed if needed, once the data from these studies are submitted and evaluated **(EPA).**

Honey Bees

Our results show that brood fed with food containing GLY traces (1.25–5.0 mg per litre of food) had a higher proportion of larvae with delayed moulting and reduced weight. Our assessment also indicates a non-monotonic dose-response and variability in the effects among colonies. Differences in genetic diversity could explain the variation in susceptibility to GLY. Accordingly, the transcription of immune/detoxifying genes in the guts of larvae exposed to GLY was variably regulated among the colonies studied. Consequently, under laboratory conditions, the response of honey bees to GLY indicates that it is a stressor that affects larval development depending on individual and colony susceptibility. **PLoS ONE 13(10): 1-19**

Glyphosate-containing products pose hazards to insects that are economically beneficial because they kill pest insects. The International Organization for Biological Control found that exposure to freshly dried Roundup killed over 50 percent of three species of beneficial insects: a parasitoid wasp, a lacewing, and a ladybug.35 Over 80 percent of a fourth species, a predatory beetle, was killed. Exposure to three sublethal concentrations of GLY (2.5, 5 and 10 mg l−1: corresponding to 0.125, 0.250 and 0.500 μg per animal) affects the homeward flight path of honeybees in an open field. We performed an experiment in which forager honeybees were trained to an artificial feeder, and then captured, fed with sugar solution containing traces of GLY and released from a novel site either once or twice. Their homeward trajectories were tracked using harmonic radar technology. We found that honeybees that had been fed with solution containing 10 mg l−1 GLY spent more time performing homeward flights than control bees or bees treated with lower concentrations. They also performed more indirect homing flights Moreover, the proportion of direct home ward flights performed after a second release from the same site increased in control bees but not in treated bees. These results suggest that, in honeybees, exposure to levels of GLY commonly found in agricultural settings impairs the cognitive capacities needed to retrieve and integrate spatial information for a successful return to the hive. Therefore, honey bee

navigation is affected by ingesting traces of the most widely used herbicide worldwide, with potential long-term negative consequences for colony foraging success.

Earthworms

Herbicide use is increasing worldwide both in agriculture and private gardens. However, our knowledge of potential side-effects on non-target soil organisms, even on such eminent ones as earthworms, is still very scarce. In a greenhouse experiment, we assessed the impact of the most widely used glyphosate-based herbicide Roundup on two earthworm species with different feeding strategies. We demonstrate, that the surface casting activity of vertically burrowing earthworms (Lumbricus terrestris) almost ceased three weeks after herbicide application, while the activity of soil dwelling earthworms (Aporrectodea caliginosa) was not affected. Reproduction of the soil dwellers was reduced by 56% within three months after herbicide application. Herbicide application led to increased soil concentrations of nitrate by 1592% and phosphate by 127%, pointing to potential risks for nutrient leaching into streams, lakes, or groundwater aquifers. These sizeable herbicide-induced impacts on agroecosystems are particularly worrisome because these herbicides have been globally used for decades **[Scientific Reports, August 2015].**

Earthworms: A study of the most common earthworm found in agricultural soils in New Zealand showed that glyphosate significantly affects growth and survival of earthworms. Repeated biweekly applications of low rates of glyphosate (1/20 of typical rates) caused a reduction in growth, an increase in the time to maturity, and an increase in mortality.

Effect on plant health

Some studies have found causal relationships between glyphosate and increased or decreased disease resistance. Exposure to glyphosate has been shown to change the species composition of endophytic bacteria in plant hosts, which is highly variable (Duke, et al., 2007 ; Rosenblueth and Romero, 2006)

As a broad-spectrum herbicide, glyphosate has potent acutely toxic effects on most plant species. However, there are other kinds of serious effects. These include effects on endangered species, reduction in the ability to fix nitrogen, increased susceptibility to plant diseases, and reduction in the activity of mycorrhizal fungi. **Journal of Pesticide Reform Volume 15, Number 4, Winter 1995**

Genetically modified crops

Some micro-organisms have a version of 5-enolpyruvoyl-shikimate-3-phosphate synthetase (EPSPS) resistant to glyphosate inhibition. A version of the enzyme that was both resistant to glyphosate and that was still efficient enough to drive adequate plant growth was identified by Monsanto scientists after much trial and error in an *Agrobacterium* strain called CP4, which was found surviving in a waste-fed column at a glyphosate production facility. This *CP4* EPSPS gene was cloned and transfected into soybeans. In 1996, genetically modified soybeans were made commercially available. Current glyphosate-resistant crops include soy, maize (corn), canola, alfalfa, sugar beets, and cotton, with wheat still under development.In 2015, 89% of corn, 94% of soybeans, and 89% of cotton produced in the United States were from strains that were genetically modified to be herbicide-tolerant (Green and Owen, 2011 ; Rashid, 2009 and Adoption of Genetically Engineered Crops in the U.S., 2015).

GOVERNMENT AND ORGANIZATION POSITIONS

European Food Safety Authority

A 2013 systematic review by the German Institute for Risk Assessment (BfR) examined more than 1000 epidemiological studies, animal studies, and *in vitro* studies. It found that "no classification and labelling for carcinogenicity is warranted" and did not recommend a carcinogen classification of either 1A or 1B. It provided the review to EFSA in January 2014 which published it in December 2014. In November 2015, EFSA published its conclusion in the Renewal Assessment Report (RAR), stating it was "unlikely to pose a carcinogenic hazard to humans". The EU was largely informed by this report when it made its decision on the use of glyphosate in November 2017.

EFSA's decision and the BfR report were criticized in an open letter published by 96 scientists in November 2015 saying that the BfR report failed to adhere to accepted scientific principles of open and transparent procedures. The BfR report included unpublished data, lacked authorship, omitted references, and did not disclose conflict-of-interest information [Charles, 2016 ; Christopher, 2015).

On April 4, 2016, Dr. Vytenis Andriukaitis, European Commissioner for Health and Food Safety, wrote an open letter to the Chair of the Board of the Glyphosate Task

at Monsanto Europe asking to publish the full studies provided to the EFSA [European Commission, 2016].

In September 2017, *The Guardian* reported that sections of the Renewal Assessment Report prepared by the BfR and used by Efsa were copy-pasted from a study done by Monsanto. Some sections of copy contained small changes such as using British spelling rather than American forms but others were copied word for word, including most of the peer-reviewed papers that were used in the report. The Guardian reported that a "Monsanto spokesperson said that Efsa allowed renewal reports to be written this way because of the large volume of toxicological studies submitted." [*The Guardian*. Retrieved, 2017].

US Environmental Protection Agency

In a 1993 review, the EPA, considered glyphosate to be noncarcinogenic and relatively low in dermal and oral acute toxicity. The EPA considered a "worst case" dietary risk model of an individual eating a lifetime of food derived entirely from glyphosate-sprayed fields with residues at their maximum levels. This model indicated that no adverse health effects would be expected under such conditions.[83] In 2015, the EPA initiated a review of glyphosate's toxicity and in 2016 reported that glyphosate is likely not carcinogenic.

In May 2019, CNN reported that the agency had reaffirmed its position that, when used according to instructions, glyphosate is not carcinogenic. Noting company emails between Monsanto and EPA executives released in 2015 which appear to suggest that an EPA official offered to kill an agency glyphosate review, CNN cited "concerns about whether Monsanto has had undue influence over regulators [Charles, Dan, 2019 ; CNN and Holly Yan, 2019).

International Agency for Research on Cancer

In March 2015, the International Agency for Research on Cancer (IARC), an intergovernmental agency forming part of the World Health Organization of the United Nations, published a summary of their forthcoming monograph on glyphosate, and classified glyphosate as "probably carcinogenic in humans" (category 2A) based on epidemiological studies, animal studies, and *in vitro* studies. It noted that there was "limited evidence" of carcinogenicity in humans for non-Hodgkin lymphoma. The IARC classifies substances for their carcinogenic potential, and "a few positive findings can be enough to declare a hazard,

even if there are negative studies, as well." Unlike the BfR, it does not conduct a risk assessment, weighing benefits against risk. [Pollack, 2015]

The BfR responded that IARC reviewed only a selection of what they had reviewed earlier, and argued that other studies, including a cohort study called *Agricultural Health Study*, do not support the classification. The IARC report did not include unpublished studies, including one completed by the IARC panel leader. The agency's international protocol dictates that only published studies be used in classifications of carcinogenicity, since national regulatory agencies including the EPA have allowed agrochemical corporations to conduct their own unpublished research, which may be biased in support of their profit motives. Monsanto called the IARC report biased and said it wanted it to be retracted. Two journalists from *Le Monde* won the 2018 European Press Prize for the "Monsanto Papers," a series of articles which described, among other things, Monsanto's lawyers' intimidation of IARC scientists after the publication of the IARC finding that glyphosate was a "probable carcinogen" in *Monograph 112*. (Gillam 2015; Glyphosate: IARC, 2018)

A 2017 review done by personnel from EFSA and BfR argued that the differences between the IARC's and EFSA's conclusions regarding glyphosate and cancer were due to differences in their evaluation of the available evidence. The review concluded that "Two complementary exposure assessments ... suggests that actual exposure levels are below" the reference values identified by the EFSA "and do not represent a public concern." In contrast, a 2016 analysis concluded that in the EFSA's *Renewal Assessment Report*, "almost no weight is given to studies from the published literature and there is an over-reliance on non-publicly available industry-provided studies using a limited set of assays that define the minimum data necessary for the marketing of a pesticide", arguing that the IARC's evaluation of *probably carcinogenic to humans* "accurately reflects the results of published scientific literature on glyphosate" (Hakim and Danny, 2017 ; Stéphane Foucart and Stéphane Horel, 2019).

In 2017, internal documents from Monsanto were made public by lawyers pursuing litigation against the company. The documents appeared to indicate that Monsanto had planned a public relations effort to discredit the IARC report, and that an opinion piece in *Forbes Magazine* challenging the report had been written by an author engaged by Monsanto who had not revealed that connection. In response, *Forbes* removed the piece. In October 2017, an article in *The Times* revealed that Christopher Portier, a scientist advising the IARC in the assessment of glyphosate and strong advocate for its classification as

possibly carcinogenic, had received consulting contracts with two law firm associations representing alleged glyphosate cancer victims that included a payment of US$160,000 to Portier. According to Geoffrey Kabat, Portier played a key role in requesting the IARC perform a review of glyphosate carcinogencity and in deliberations that result in the IARC's conclusion that glyphosate was carcinogenic. Following these reports of Portier's actions, the IARC's final report was also found to have undergone significant changes compared to an interim report through removal of text saying glyphosate was not carcinogenic and to strengthening claims of carcinogenicity. During deposition, Portier said the interim report originally did conclude "limited evidence of animal carcinogenicity." but denied knowing when the text was changed to "sufficient evidence of animal carcinogenicity". [Kelland, Kate, 2019 ; Reuters, 2016]

California Office of Environmental Health Hazard Assessment

After the California Office of Environmental Health Hazard Assessment (OEHHA) announced, in March 2015, plans to have glyphosate listed as a known carcinogen based on the IARC assessment, Monsanto started a case against OEHHA and its acting director, Lauren Zeise, in 2016, but lost the suit in March 2017 [Kelland, Kate, 2019 ; Reuters, 2016]. Glyphosate was listed as "known to the State of California to cause cancer" in 2017. As part of an ongoing case, an injunction was issued prohibiting California from enforcing carcinogenicity labeling requirements for glyphosate stating that arguments by California "[do] not change the fact that the overwhelming majority of agencies that that have examined glyphosate have determined it is not a cancer risk."

European Chemicals Agency

On March 15, 2017 the European Chemicals Agency (ECHA) announced recommendations proceeding from a risk assessment of glyphosate performed by ECHA's Committee for Risk Assessment (RAC). Their recommendations maintained the current classification of glyphosate as a substance causing serious eye damage and as a substance toxic to aquatic life. However, the RAC did not find evidence implicating glyphosate to be a carcinogen, a mutagen, as toxic to reproduction, nor as toxic to specific organs.

European Union

In April 2014, the legislature of the Netherlands passed legislation prohibiting sale of glyphosate to individuals for use at home; commercial sales were not affected. In June 2015,

the French Ecology Minister asked nurseries and garden centers to halt over-the-counter sales of glyphosate in the form of Monsanto's Roundup. This was a nonbinding request and all sales of glyphosate remain legal in France until 2022, when it was planned to ban the substance for home gardening. However, more recently the French parliament decided to not to impose a definitive date for such a ban. In January 2019, "the sale, distribution, and use of Roundup 360 [wa]s banned" in France. Exemptions for many farmers were later implemented, and a curb of its use by 80% for 2021 is projected. [Weedkiller Roundup banned in France after court ruling, 2019 ; Arthur Nelson, 2016]

A vote on the relicencing of glyphosate in the EU stalled in March 2016. Member states France, Sweden, and the Netherlands objected to the renewal. A vote to reauthorize on a temporary basis failed in June 2016 but at the last-minute the license was extended for 18 months until the end of 2017. On 27 November 2017, a majority of eighteen EU member states voted in favor of permitting the use of herbicide glyphosate for five more years. A qualified majority of sixteen states representing 65% of EU citizens was required. The German Minister of Agriculture, Christian Schmidt, unexpectedly voted in favor while the German coalition government was internally divided on the issue which usually results in Germany abstaining.

In December 2018, attempts were made to reopen the decision to license the weed-killer. These were condemned by Conservative MEPs, who said the proposal was politically motivated and flew in the face of scientific evidence. In March 2019, the European Court of Justice (ECJ) ordered the European Food Safety Authority (EFSA) to release all carcinogenicity and toxicity pesticide industry studies on glyphosate to the general public. [European Court of Justice orders public release of industry glyphosate studies', 2019 ; Briner, 2018].

In March 2019, the Austrian state of Carinthia outlawed the private use of glyphosate in residential areas while the commercial application of the herbicide is still permitted for farmers. The use of glyphosate by public authorities and road maintenance crews was already halted a number of years prior to the current ban by local authorities. In June 2019, Deutsche Bahn and Swiss Federal Railways announced that glyphosate and other commonly used herbicides for weed eradication along railway tracks will be phased out by 2025, while more environmentally sound methods for vegetation control are implemented. In July 2019, the Austrian parliament voted to forbid glyphosate [Austria Staff, Centralamericadata.com].

Other countries

In September 2013, the Legislative Assembly of El Salvador approved legislation to ban 53 agrochemicals, including glyphosate; the ban on glyphosate was set to begin in 2015. In May 2015, the President of Sri Lanka banned the use and import of glyphosate, effective immediately. However, in May 2018 the Sri Lankan government decided to re-authorize its use in the plantation sector. (Legislative Assembly of El Salvador, 2013 ; Glyphosate ban lifted for tea, rubber industries).

In May 2015, Bermuda blocked importation on all new orders of glyphosate-based herbicides for a temporary suspension awaiting outcomes of research. In May 2015, Colombia announced that it would stop using glyphosate by October 2015 in the destruction of illegal plantations of coca, the raw ingredient for cocaine. Farmers have complained that the aerial fumigation has destroyed entire fields of coffee and other legal produce. In April 2019, Vietnam's Ministry of Agriculture and Rural Development banned the use of glyphosate throughout the country. The ministry had begun reviewing the use of the chemical in 2016. (Viet Nam bans weed killer ingredient glyphosate, 2019).

Lawsuits claiming liability for cancer

In June 2018, Dewayne Johnson, a 46-year-old former California school groundskeeper who is dying of non-Hodgkin lymphoma, took Monsanto (which had been acquired by Bayer earlier that month) to trial in San Francisco County superior court, alleging that it has spent decades hiding the cancer-causing dangers of its Roundup herbicides. The judge ordered that jurors be allowed to consider both scientific evidence related to the cause of Johnson's cancer and allegations that Monsanto suppressed evidence of the risks, with possible punitive damages. In August 2018, the jury awarded Johnson US$289 million in damages. Monsanto said they would appeal, saying they were confident that glyphosate does not cause cancer when used appropriately. In November 2018, the award was reduced to 78 million on appeal. (Weedkiller glyphosate doesn't cause cancer' 2018).

In August 2018, the potential for additional cases was estimated at up to 4,000. Bayer announced in April 2019 that over 13,000 lawsuits related to Roundup had been launched in the US. In March 2019, a man was awarded $80 million in a lawsuit claiming Roundup was a substantial factor in his cancer, resulting in Costco stores discontinuing sales. In July 2019, U.S. District Judge Vince Chhabria reduced the settlement to $26 million. Chhabria stated that a punitive award was appropriate because the evidence "easily supported a conclusion

that Monsanto was more concerned with tamping down safety inquiries and manipulating public opinion than it was with ensuring its product is safe." Chhabria stated that there is evidence is on both sides concerning whether glyphosate causes cancer and that the behavior of Monsanto showed "a lack of concern about the risk that its product might be carcinogenic (Johnson and Stephen, 2019).

On 13 May 2019 a jury in California ordered Bayer to pay a couple $2 billion in damages after finding that the company had failed to adequately inform consumers of the possible carcinogenicity of Roundup. On July 26, 2019, an Alameda County judge cut the settlement to $86.7 million, stating that the judgement by the jury exceeded legal precedent. Using litigation discovery emails it was later revealed that in 2015 when Monsanto was discussing papers they wanted to see published to counter the expected IARC glyphosate results they wrote in an email, "An option would be to add Greim and Kier or Kirkland to have their names on the publication, but we would be keeping the cost down by us doing the writing and they would just edit & sign their names so to speak. Recall that is how we handled Williams Kroes & Munro, 2000."

CASE STUDY

The acute toxicity of glyphosate products to humans was first widely publicized by physicians in Japan who studied 56 cases of Roundup poisoning. Most of the cases were suicides or attempted suicides; nine cases were fatal. Symptoms of acute poisoning in humans included gastrointestinal pain, vomiting, excess fluid in the lungs, pneumonia, clouding of consciousness, and destruction of red blood cells. They calculated that the mean amount ingested in the fatal cases was slightly more than 200 milliliters (about 3/4 of a cup). They believed that POEA was the cause of Roundup's toxicity. More recent reviews of glyphosate poisoning incidents have found similar symptoms, as well as lung congestion or dysfunction, erosion of the gastrointestinal tract, abnormal electrocardiograms, massive gastrointestinal fluid loss, low blood pressure, and kidney damage or failure.

Where is Glyphosate Banned?

A number of cities, counties, states and countries throughout the world have taken steps to either restrict or ban glyphosate, the active ingredient in Monsanto's Roundup weed killer.

The following countries have issued outright bans on glyphosate, imposed restrictions or have issued statements of intention to ban or restrict glyphosate-based herbicides, including Roundup, over health concerns and the ongoing Roundup cancer litigation:

- **Argentina:** In 2015, more than 30,000 health care professionals advocated for a glyphosate ban following the International Agency for Research on Cancer's (IARC) report on glyphosate, which concluded the chemical is probably carcinogenic to humans. More than 400 towns and cities in Argentina have passed measures restricting glyphosate use.

- **Australia:** Numerous municipalities and school districts throughout the country are currently testing alternative herbicides in an effort to curtail or eliminate glyphosate use. Many use steam technology for weed control on streets and in other public areas.
- Following a series of massive jury verdicts in Roundup cancer lawsuits in the United States, the Australian state of Victoria launched its own review of glyphosate. Two councils in Sydney have either banned or are in the process of banning glyphosate use, and eight other councils are reviewing the chemical.

- **Austria**: In June of 2019, Austria announced that it planned to ban glyphosate within the year. Leader of the Social Democrats, Pamela Rendi-Wagner, said she is "pleased" that her party's long-standing effort to ban glyphosate in Austria would "finally pay off" now that her party's motion had a majority in the Austrian parliament. The measure to ban glyphosate passed in July of 2019. The Austria glyphosate ban will take effect on January 1, 2020.

- **Bahrain:** According to Oman's Ministry of Agriculture, Bahrain and five other countries in the Gulf Cooperation Council (GCC) have banned glyphosate.

- **Belgium:** Banned the individual use of glyphosate. In 2017, Belgium voted against relicensing glyphosate in the EU. The country was also one of six EU member states to sign a letter to the EU Commission calling for "an exit plan for glyphosate…" The city of Brussels banned the use of glyphosate within its territory as part of its "zero pesticides" policy.

- **Bermuda:** Outlawed private and commercial sale of all glyphosate-based herbicides. In 2017, the government relaxed its ban on glyphosate, allowing the Department of

Environment and Natural Resources to import restricted concentrations of glyphosate for managing roadside weed overgrowth.

- **Brazil:** In August of 2018, a federal judge in Brasilia ruled that new products containing glyphosate could not be registered in the country. Existing regulations concerning glyphosate were also suspended, pending a reevaluation of toxicological data by Anvisa, the country's health agency.

- In September of 2018, a Brazilian court overturned the federal judge's ruling. September marks Brazil's first month of soybean planting. The country is the largest exporter of soybeans in the world, and as such, has become heavily reliant on agrochemicals. Anvisa issued a statement following the court's decision to overturn the ruling, saying it will take necessary legal and technical steps in response. Further. Brazil's Solicitor General's office has said it is preparing an appeal to the court decision with support from the Agriculture Ministry. Brazil's health agency concluded a re-evaluation of glyphosate in February of 2019. Based on the agency's findings, a blanket ban of glyphosate in Brazil is unlikely.

- **Canada:** Eight out of the 10 provinces in Canada have some form of restriction on the use of non-essential cosmetic pesticides, including glyphosate. Vancouver has banned public and private use of glyphosate, aside from the treatment of invasive weeds. In June of 2019, New Brunswick officials announced that the province would reduce glyphosate spraying in certain areas with the promise that more regulation will follow.

- **Colombia:** In 2015, Colombia outlawed the use of glyphosate to destroy illegal plantations of coca, the raw ingredient for cocaine, out of concern that glyphosate causes cancer. In March of 2019, President Ivan Duque asked for the judicial ban on aerial glyphosate spraying to be lifted. However, in July of 2019, the court maintained the judicial ban on glyphosate, ruling that the government has to prove that glyphosate is not harmful to human health and the environment in order for the ban to be lifted.

- **Czech Republic:** Agriculture Minister Miroslav Toman said the country will limit glyphosate use starting in 2019. Specifically, the Czech Republic will ban glyphosate as a weedkiller and drying agent.

- **Denmark:** The Danish Working Environment Authority declared glyphosate to be carcinogenic and has recommended a change to less toxic chemicals. Aalborg, one of the largest cities in Denmark, issued private-use glyphosate ban in September of 2017. In July of 2018, the Danish government implemented new rules banning the use of glyphosate on all post-emergent crops to avoid residues on foods.

- **El Salvador:** In 2013, the country adapted a law banning glyphosate over links to deadly kidney disease. However, by 2016, the legislation appeared to stall.

- **France:** French authorities banned the sale, distribution and use of Roundup 360 in early 2019. In May of 2019, French Agriculture Minister Didier Guillaume announced that France would eliminate the use of glyphosate by 2021 with limited exceptions. Some 20 mayors throughout the country have banned glyphosate in their municipalities.

- **Germany:** Environment Minister Svenja Schulze announced in September 2019 that Germany will ban glyphosate by 2023. The ban, agreed to by the Cabinet, includes a "systemic reduction strategy" that will prohibit glyphosate spraying in domestic gardens and at the edges of farmland. Certain retail stores in Germany have already pulled glyphosate-based herbicides like Roundup from shelves.

- **Greece:** Greece was one of nine EU countries to vote against relicensing glyphosate in November of 2017. The country was also one of six EU member states to sign a 2018 letter to the European Commission calling for "an exit plan for glyphosate…" According to Greek Minister of Agricultural Development Evangelos Apostolou, "[i]t is our duty to push in the direction of risk management, in the interests of consumers, producers and the environment." In March of 2018, the Greek government approved a five-year license for Monsanto's Roundup against the wishes of Greek environmentalists.

- **India:** In October of 2018, the government of Punjab banned the sale of glyphosate in the state. "All pesticide manufacturers, marketers and dealers in the State shall not sell glyphosate formulations-concentrations with immediate effect. The licensing authorities have been asked to take necessary steps for removal of entries for glyphosate from the licenses issued by them," said State Agriculture Secretary K.S.

Pannu. In February of 2019, the Indian state of Kerala issued a ban on the sale, distribution and use of glyphosate.

- **Italy:** Italy's Ministry of Health placed a number of restrictions on glyphosate use. Italian legislators have also raised concerns about glyphosate safety, and have come out against relicensing the herbicide in the European Union. In 2016, the Italian government banned the use of glyphosate as a pre-harvest treatment and placed restrictions on glyphosate use in areas frequented by the public. In November of 2017, Italy was one of seven EU nations to vote against relicensing glyphosate.

- **Kuwait:** According to Oman's Ministry of Agriculture, Kuwait and five other countries in the Gulf Cooperation Council (GCC) issued glyphosate bans.
- **Luxembourg:** One of Luxembourg's largest supermarket chains removed glyphosate from its shelves following the release of the IARC glyphosate report. Luxembourg was one of nine EU countries to vote against relicensing glyphosate in November of 2017, and in early 2018, the country signed a letter to the EU Commission calling for "an exit plan for glyphosate…"

- **Malawi:** In April 2019, Malawi's Principal Secretary of the Ministry of Agriculture, Irrigation and Water Development told the country's National newspaper that import licenses for glyphosate-based herbicides like Monsanto's Roundup would be suspended immediately.

- **Malta:** In July of 2019, Malta banned the use of glyphosate in public spaces. The spraying of glyphosate will not be allowed on roadsides or near schools, among other places.

- **Netherlands:** Banned all non-commercial use of glyphosate.

- **New Zealand:** The cities of Auckland and Christchurch passed resolutions to reduce the usage of chemicals for weed and pest control in public places. The Physicians and Scientists for Global Responsibility, a New Zealand charitable trust, called for a glyphosate ban in 2015.

- **Oman:** Eng Saleh al Abri, director general of agricultural development in Oman's Ministry of Agriculture and Fisheries (MoAF), told a reporter that glyphosate "hasn't been available in Oman since 2016." Eng Abri added, "This active ingredient has

been banned throughout the GCC (Gulf Cooperation Council) since last year." In addition to Oman, the GCC includes Saudi Arabia, Qatar, Kuwait, Bahrain, and the United Arab Emirates (UAE).

- **Portugal:** Prohibits the use of glyphosate in all public spaces. The president of the Portuguese Medical Association has also called for a worldwide ban of glyphosate.

- **Qatar:** According to Oman's Ministry of Agriculture, Qatar and five other countries in the Gulf Cooperation Council (GCC) have banned glyphosate.

- **St. Vincent and the Grenadines:** Acting on advice from their Pesticides Board, the Caribbean country placed an immediate suspension on the import of glyphosate-based herbicides.

- **Saudi Arabia:** Issued a glyphosate ban along with five other countries in the Gulf Cooperation Council (GCC).

- **Scotland:** Aberdeen cut back its use of herbicides and Edinburgh's City Council voted to phase out glyphosate. In November of 2017, five of Scotland's six EU parliamentarians voted in favor of a motion that would phase out glyphosate by 2022.

- **Slovenia:** Slovenia was one of six EU member states to sign a 2018 letter to the European Commission citing "concerns" about the risks associated with glyphosate. The letter called upon the Commission to introduce "an exit plan for glyphosate…"

- **Spain:** According to Kistiñe Garcia of the Spanish NGO, Ecologistas en Acción, Barcelona, Madrid, Zaragoza and the region of Extremuda have decided to ban glyphosate. The regions of La Rioja (major Spanish wine region) and Aragon have also approved motions against endocrine-disrupting chemicals, which includes glyphosate.

- **Sri Lanka:** Sri Lanka was the first country to issue a nationwide ban on glyphosate. However, in 2018, the government decided to lift the ban due to crop losses and overgrowing weeds.

- **Sweden:** Raised concerns about glyphosate safety and has pushed against relicensing the herbicide in the EU. In 2017, the Swedish Chemicals Agency (SCA) announced it

was planning to tighten rules on private use of plant protection products. Under the plan, private users would only be allowed to use products containing "low-risk substances." According to the SCA, glyphosate is an example of an active substance not expected to be included among low-risk substances, meaning in due time, private consumers may not be permitted to use herbicides containing glyphosate.

- **Switzerland:** Concerned about public well-being, the Swiss supermarket chains Migros and Coop removed glyphosate-based products from their shelves due to health risks. In 2017, the Green party put forth a plan to ban glyphosate in Switzerland. The proposed plan was rejected by the Federal Council, Switzerland's executive.

- **Thailand:** In August 2019, Deputy Agriculture Minister Mananya Thaiseth ceased licensing extensions for three hazardous farm chemicals, including glyphosate. According to Thaiseth, glyphosate will be banned by the end of 2019.

- **United Arab Emirates:** Issued a glyphosate ban along with five other countries in the Gulf Cooperation Council.

- **United Kingdom:** Following the landmark $289 million Monsanto Roundup verdict on Aug. 10, 2018, Homebase, one of the UK's largest DIY retailers, announced that it would review the sale of Roundup and Ranger Pro. However, according to the Sun, Homebase and other major retailers still stock the weed killers for sale.

The following boroughs and townships have issued bans or restrictions on pesticides and herbicides, including glyphosate:

- Brighton
- Bristol
- Bury (ban in children's play areas)
- Croydon
- Derry City (Northern Ireland)
- Frensham
- Frome
- Glastonbury
- Hammersmith & Fulham

- Lewes
- Midlothian (Scotland)
- North Somerset
- Trafford
- Wadebridge

- **Vietnam:** Following the jury verdict in *Hardeman v. Monsanto Co.*, Vietnam announced that it would ban glyphosate imports. According to Hoang Trung, Director of the Plant Protection Department under the Ministry of Agriculture and Rural Development, "the removal of this substance from the list of pesticides allowed to be used in Vietnam will be done in the near future."

Why is Glyphosate Banned?

Most of the glyphosate restrictions or bans throughout the world were introduced following the 2015 IARC report on glyphosate. The IARC report concluded that glyphosate is a "probable human carcinogen." According to the report, the cancers most associated with glyphosate exposure were found to be non-Hodgkin lymphoma and other hematopoietic cancers. The report further concluded that glyphosate exposure caused DNA and chromosomal damage in human cells, as well as genotoxic, hormonal and enzymatic effects in mammals.

Other glyphosate studies have linked the chemical to a number of health issues, including, but not limited to ADHD, Alzheimer's Disease, Autism, Birth Defects, various forms of cancer, Celiac Disease, Colitis, Heart Disease, Inflammatory Bowel Syndrome, Kidney Disease, Liver Disease, and Parkinson's Disease.

Is Glyphosate Banned in Europe?

As you can see above, some individual countries have introduced legislation to ban or restrict private sales of glyphosate, or restrictions on spraying glyphosate in public spaces. As for the whole of the European Union (EU), glyphosate is not currently banned.

However, EU public opinion is leaning in favor of a glyphosate ban. In a 2016 poll of the five largest EU countries, over 66 percent of respondents said they favored a glyphosate ban. Over 1.3 million people signed a petition in 2017 calling for a European ban of

glyphosate. That public pressure caught the attention numerous Members of European Parliament, who have cited the petition as the foundation for instituting an EU ban.

In November of 2017, EU member states narrowly voted to relicense glyphosate for a period of five years. The vote was not without controversy; German Agriculture Minister Christian Schmidt (CSU) entered a 'yes' vote for his country without consulting with German Chancellor Angela Merkel (CDU) on the matter. His unilateral vote disregarded Germany's Environment Minister, who had instructed Schmidt to abstain from voting. With Germany's vote, the measure narrowly passed and glyphosate received a new license.
Following the scandal, six EU countries sent a letter to the European Commission, calling for an exit plan for glyphosate. France and Italy have stated they will carry out glyphosate bans by 2020, and Germany announced in 2018 that it will also issue a glyphosate ban.

In January of 2019, a European Parliament report found that EU regulators based their decision to relicense glyphosate on an assessment that was plagiarized from a coalition of pesticide companies, including Monsanto.

The EU Parliament report investigated claims that Germany's Federal Institute for Risk Assessment (BfR) copied and pasted large sections of a pesticide industry assessment of glyphosate literature in its own assessment. The BfR report concluded that classifying glyphosate as a carcinogen is not warranted. The European Food Safety Authority (EFSA), which relied upon the BfR report, also found that glyphosate is safe for humans and the environment.

Following the release of the EU Parliament report, an EU court ruled that EFSA should publicize glyphosate studies used for its assessments.

Is Glyphosate Banned in the United States?

Despite the IARC report's 2015 conclusion that glyphosate is a probable human carcinogen, the U.S. Environmental Protection Agency (EPA) maintains that glyphosate is not likely to be carcinogenic to humans. As such, glyphosate is not banned by the U.S. government; Roundup and other glyphosate-based herbicides are readily available for purchase throughout the country.

However, the EPA is a captured agency, meaning it is dominated by the industry it presumably regulates. Internal company documents now public in the Monsanto

Papers demonstrate that EPA prioritizes the interests of corporations like Monsanto or political groups over the interests of the public it is charged with protecting.

"The EPA has got it wrong on glyphosate. We have study after study after study showing that it in fact, does cause a specific type of cancer called lymphoma. And we see it happening in thousands and thousands of people across the country. Currently, this Administration and this EPA will not take action against Monsanto. We've seen the internal documents, the text messages, the emails between senior EPA officials and Monsanto employees. And the simple fact is they know that this EPA will not take adverse action against them. It is a travesty that this truth about it causing cancer and this awareness that we are trying to raise has to be done in the context of litigation. We only exist, these lawsuits only exist, because the EPA has failed the American public for 45 years and Monsanto is allowed to get away with reckless conduct with, essentially, impunity...this agency essentially does not work for the American public but works for industry. The fact that the White House is telling Monsanto, 'We have your back.' I mean this just tells us that we are going to have to keep fighting this fight and that we are not going to get any support or help from the public agencies that, ironically, are supposed to be protecting the public health."

Brent Wisner, Roundup Cancer Attorney

Is Glyphosate Banned in California?

California has not issued a statewide ban on glyphosate. However, on July 7, 2017, California became the first state in the nation to issue a warning on glyphosate by adding the chemical to the state's Proposition 65 list of chemicals and substances known to cause cancer.

California's decision to warn consumers about glyphosate was pursuant to the requirements of the Safe Drinking Water and Toxic Enforcement Act, better known as California Proposition 65, a ballot initiative approved by voters in 1986 to address toxic chemical exposure concerns. Prop 65 requires California to publish a list of chemicals known to cause cancer, birth defects or other reproductive harm.

In 2019, University of California President Janet Napolitano announced that glyphosate would be temporarily banned on all 10 UC campuses, citing "concerns about possible human health and ecological hazards, as well potential legal and reputational risks associated with this category of herbicides."

Arizona

- **Tucson, Arizona** – Created an organics-first policy for controlling weeds on city property.

California

- **Alameda County, California** – The East Bay Regional Park District, a special district operating regional parks in Alameda and Contra Costa Counties, banned glyphosate around picnic and play areas effective July 2019. EBRPD plans to formally ban Roundup use in its parks by the end of 2020. EBRPD manages 73 parks and 55 miles of shoreline.
- **Arcata, California** – Initiated a pesticide reduction plan that urges pesticides to only be used as a last resort.
- **Belvedere, California** – Passed municipal ordinance initiating Integrated Pest Management program that restricts toxic pesticide use and urges pesticide use as last resort.
- **Benicia, California** – City decided to go glyphosate-free following the verdict in Johnson v. Monsanto Co.
- **Berkeley, California** – Implemented pest management program to minimize or eliminate the use of pesticides. The city has not used glyphosate since the 1970s, according to spokesman Matthai Chakko.
- **Burbank, California** – City Council members voted to discontinue the use of Roundup in city parks for one year, and Burbank Unified School District will no longer use the herbicide due to cancer concerns.
- **Cambria, California** – North Coast school board trustees formally proposed a ban on glyphosate for all school properties.
- **Carlsbad, California** – The City Council voted unanimously to adopt a policy that makes organic pesticides the preferred method for killing weeds. "Asked to choose between aesthetics and public health…I'm going to choose public health every time," said Councilwoman Cori Schumacher.
- **Concord, California** – The Mount Diablo Unified School District unanimously voted to ban glyphosate use on school property.
- **Contra Costa County, California** – The East Bay Regional Park District, a special district operating regional parks in Alameda and Contra Costa Counties, banned glyphosate around picnic and play areas effective July 2019. EBRPD plans to

formally ban Roundup use in its parks by the end of 2020. EBRPD manages 73 parks and 55 miles of shoreline.

- **Corte Madera, California** – Passed ordinance calling for Integrated Pest Management (IPM) program restricting highly toxic pesticides, while also urging for pesticide use to be a last resort.
- **Costa Mesa, California** – City council adopted an organics-first Integrated Pest Management (IPM) policy.
- **Davis, California** – Passed ordinance implementing Integrated Pest Management (IPM) program designed to reduce the use of pesticides. Some city parks do not allow the use of glyphosate.
- **Encinitas, California** – Banned the use of Roundup and other glyphosate-based weed killers in city parks.
- **Fairfax, California** – Passed municipal ordinance restricting use of toxic pesticides on public property in favor of alternative methods.
- **Fresno, California** – After hearing from concerned parents and employees, Fresno Unified School District is investigating the use of alternative herbicides that do not contain glyphosate, citing health risks.
- **Greenfield, California** – Adopted a resolution to "halt all use of the carcinogenic weed killer Roundup and replace it with 'greener' alternatives."
- **Irvine, California** – City Council passed resolution to cease spraying Roundup and other chemicals on public parks, streets and playgrounds.
- **Laguna Hills, California** – Passed a resolution to test an organics-only pesticide program on two parks.
- **Lodi, California** –The city decided to ban the use of Roundup within 25 feet of playgrounds.
- **Long Beach, California** – Citing the landmark $289 million verdict in Johnson v. Monsanto Co., Long Beach Parks & Recreation Director Gerardo Mouet announced an immediate halt on the spraying of Roundup in Long Beach Parks.
- **Los Angeles County, California** – The Los Angeles County Board of Supervisors issued a moratorium on glyphosate-based herbicides, including Roundup weed killer. In July 2019, the LA County Board of Supervisors formally banned Roundup.
- **Malibu, California** – The city may implement an Earth Friendly Management Policy (EFMP) to avoid the use of pesticides and other chemicals.

- **Marin County, California** – The county stopped using glyphosate, the active ingredient in Monsanto's Roundup weed killer, on all county-maintained parks, landscaping, playgrounds, walkways and parking areas.
- **Mill Valley, California** – Passed ordinance initiating Integrated Pest Management program that restricts toxic pesticide use and urges pesticide use as last resort.
- **Morgan Hill, California** – Instituted a pilot program at a city park to assess the possibility of eliminating the use of herbicides.
- **Napa, California** – A policy announced in March of 2019 banned glyphosate use on city property, completing a phase-out campaign that started three years ago.
- **Novato, California** – Following the $289 million Monsanto verdict, Novato Mayor Josh Fryday said the city will no longer use Roundup weed killer.
- **Oakland, California** – Passed ordinance initiating Integrated Pest Management program that restricts toxic pesticide use and promotes pesticide use as last resort. On Sept. 1, 2018, the city formally halted the use of Roundup. Alameda County is reviewing its chemical spraying practices.
- **Orange County, California** – OC Parks banned the use of glyphosate on and around playgrounds, picnic shelters, trails and campgrounds. However, glyphosate remains in use on off-trail invasive weeds.
- **Oxnard, California** – The Oxnard School District board voted to ban Roundup use on campuses.
- **Palo Alto, California** – Pest management program calls for Integrated Pest Management that restricts pesticide use in favor of less harmful methods.
- **Petaluma, California** – City officials are considering a ban on glyphosate for use in public parks.
- **Richmond, California** – Issued an ordinance to ban the use of glyphosate for all weed abatement activities conducted by the city.
- **San Anselmo, California** – Passed city resolution promoting an Integrated Pest Management program restricting the use of toxic pesticides. The program only allows pesticide use as a last resort.
- **San Francisco, California** – Restricts the use of toxic pesticides on public property in favor of alternative, organic methods.
- **San Juan Capistrano, California** – Implemented an organics-first policy to control weeds in city parks and open spaces.

- **San Lorenzo Valley, California** – The San Lorenzo Valley Water District voted 4-1 for a permanent ban of glyphosate pesticide use by the district.
- **San Luis Obispo, California** – San Luis Coastal Unified School District banned all pesticides, including Roundup, on school properties in 2018. Coast Unified School District banned Roundup in the summer of 2019.
- **Santa Barbara, California** – The Santa Barbara Unified School District Board of Education voted to ban glyphosate spraying at all district schools.
- **Santa Rosa, California** – Banned the use of Roundup at city parks.
- **Sonoma, California** – Banned glyphosate use on all city-owned property.
- **Thousand Oaks, California** – City instituted a ban on glyphosate use on public golf courses.
- **Watsonville, California** – City council voted unanimously to ban Roundup use on city property.
- **Woodland, California** – Woodland Joint Unified School District suspended the use of Roundup on school campuses.

Colorado

- **Boulder, Colorado** – Banned Roundup for use on city parks.
- **Durango, Colorado** – Instituted an Organically Managed Lands program to minimize the use of synthetic fertilizers and pesticides.

Connecticut

- **Middletown, Connecticut** – Passed ordinance banning toxic pesticides and herbicides on municipally-owned fields, parks and other property.

A growing number of Connecticut towns, including Branford, Cheshire, Granby, Essex, Greenwich, Manchester, Oxford, Pine Grove, Plainville, Roxbury, Watertown, and Woodbridge have adopted bans or restrictions on glyphosate use. The state also has Public Act 09-56 to eliminate the use pesticides in K-8 schools.

Florida

The Florida Fish and Wildlife Conservation Commission ceased using aquatic herbicides, glyphosate chief among them, anywhere in state waters, while the agency gathers public input.

- **Fort Myers Beach, Florida** – The city has decided to ban Roundup.
- **Key West, Florida** – Key West City Commission banned the use of Roundup on city-owned property, citing a $2.055 billion jury verdict in California.

- **Martin County, Florida** – The local government instituted a Roundup ban that applies to all county employees and contractors working on county projects.
- **Miami, Florida** – Announced a city-wide ban on glyphosate-based herbicides in February of 2019.
- **Miami Beach, Florida** – Passed a resolution banning the use of glyphosate weed killers for landscaping and maintenance work on city-owned property.
- **North Miami, Florida** – City Council approved a plan calling for the gradual reduction of pesticide use on city property and a study on alternative pesticides.
- **Satellite Beach, Florida** – City Council unanimously approved a resolution that bans the city and its contractors from using glyphosate-based herbicides, including Monsanto's Roundup.
- **Stuart, Florida** – City commissioners voted to ban glyphosate, calling for an integrated pest control plan that reduces the use of glyphosate with the ultimate goal of eliminating chemicals.

Hawaii

In February of 2018, a series of bills moved ahead in the legislature that would regulate pesticides, including Roundup weed killer.

Illinois

- **Chicago, Illinois** – The city stopped spraying glyphosate in public spaces.
- **Evanston, Illinois** – Evanston decided to go pesticide-free in 2010. Glyphosate is banned from use on city property, parks and schools.
- **Franklin Park, Illinois** – Passed resolution promoting an Integrated Pest Management (IPM) policy that restricts highly toxic pesticides and urges for pesticides to be considered as a last resort.
- **Naperville, Illinois** – Created the Sustainable Parks Initiative, which uses organic products and sustainable practices for weed control.
- **Urbana, Illinois** – Adopted the Midwest Grows Green natural lawn care initiative to eliminate synthetic lawn pesticides on city parks.

Iowa

- **Dubuque, Iowa** – City instituted a ban on glyphosate use in public parks.
- **Story County, Iowa** – Eliminated the use of chemical pesticides in six of its mowed turf areas.

Kansas

- **Lawrence, Kansas** – Implemented Integrated Pest Management (IPM) program designed to reduce pesticide use.
- **Wichita, Kansas** – Initiated pilot program that limits or eliminates pesticide use.

Maine

Dozens of cities and townships in Maine have adopted local ordinances restricting or banning pesticides and herbicides.

- **Portland, Maine** – Banned synthetic pesticides in March of 2019. Private property owners may only use organic treatments on lawns and gardens. No pesticides may be used within 75 feet of a water body or wetland.
- **South Portland, Maine** – Passed a pesticide plan that discourages property owners from using certain pesticides and herbicides.

Maryland

- **Greenbelt, Maryland** – Adopted Sustainable Land Care policy for public lands calling for limited use of pesticides.
- **Hyattsville, Maryland** – Passed ordinance prohibiting the use of toxic pesticides on public property in favor of alternative, organic methods
- **Montgomery County, Maryland** – County Council voted to ban the use of cosmetic pesticides on private lawns. In December 2018, Montgomery County Parks announced that it would discontinue the use of glyphosate in parks.
- **Takoma Park, Maryland** – Placed restriction on cosmetic pesticides for lawn care on public and private property.

Massachusetts

- **Chatham, Massachusetts** – Passed an order banning glyphosate use in parks, athletic fields, mulch beds and walkways.
- **Eastham, Massachusetts** – Local ordinance requires town employees to receive a permit for use of registered pesticides and prohibits the use of highly-toxic pesticides.
- **Falmouth, Massachusetts** – Issued a yearlong moratorium on glyphosate use.
- **Marblehead, Massachusetts** – Created Organic Pest Management program to phase out pesticides and herbicides.
- **Warwick, Massachusetts** – A measure to ban Monsanto's Roundup passed at a Special Town Meeting. The ban does not allow people to spray glyphosate on any land within the town.

- **Wellesley, Massachusetts** – Wellesley banned all pesticides in 2011. Glyphosate is restricted from being sprayed on athletic fields and any city-owned property. The chemical can be used in limited emergency weed control situations.

Minnesota

- **Minneapolis, Minnesota** – Commissioners of the Minneapolis Parks and Recreation Board decided to eliminate all glyphosate-based products from being used in neighborhood parks. In October of 2018, the Park Board's Operations & Environment Committee voted to extend the glyphosate ban to the entire Minneapolis park system.
- **Rochester, Minnesota** – The Parks & Recreation Department initiated a pesticide-free pilot project for city parks.

Nevada

- **Reno, Nevada** – The city initiated a pesticide free pilot program.

New Hampshire

- **Dover, New Hampshire** – Passed resolution calling for Organic Land Management. City utilizes least toxic compounds only when necessary.
- **Portsmouth, New Hampshire** – Passed resolution eliminating the use of toxic pesticides on public property in favor of alternative, organic methods.

New Mexico

- **Bernalillo County, New Mexico** – The County Commission voted to ban the use of Roundup on county properties by 2020.
- **Las Cruces, New Mexico** – The Las Cruces City Council voted to ban Roundup and its principal ingredient, glyphosate, for pest control on city property. The ban is scheduled to take effect once the city's glyphosate supply is exhausted.
- **Taos County, New Mexico** – Taos County Commissioners are considering the possibility of banning all pesticides, including glyphosate.

New Jersey

New Jersey has State and local ordinances encouraging Integrated Pest Management programs to eliminate or drastically reduce the use of pesticides. At least 15 city school districts and over a dozen other parks and recreation departments in the state have enacted IPM programs.

New York

In January of 2019, New York State Senator Brad Hoylman (27th District) sponsored a bill in the New York State Senate that would prohibit the sale and distribution of products containing glyphosate. Updates on the legislation can be found here.

In April of 2019, two New York City council members introduced legislation to ban glyphosate use in parks and other public spaces.

New York Park and Recreation Department has measures to eliminate or reduce pesticide and herbicide use in areas under its control.

- **New Paltz, New York** – The use of toxic pesticides and herbicides by city employees or by private contractors is forbidden on all city-owned lands.
- **Rockland County, New York** – Created a Non-Toxic Pesticide program, mandating the use of natural, non-toxic, or as a last resort with prior approval, the least toxic pesticide use.
- **Westchester County, New York** – Enacted a law for pesticide-free parks.

North Carolina

- **Carrboro, North Carolina** – The city of Carrboro has restricted glyphosate use since 1999. Under the terms of the ban, glyphosate cannot be sprayed in public parks, schools and town buildings or properties. The city will only allow glyphosate to be sprayed under limited circumstances.

Ohio

- **Cuyahoga County, Ohio** – Local ordinance prohibits the use of pesticides on county-owned land. and established the adoption of an Integrated Pest Management program for county-owned properties.
- **South Euclid, Ohio** – Passed ordinance prohibiting toxic pesticides on public grounds in favor of alternative, organic pest control methods unless permitted by an Environmental Review Board.

Oregon

- **Eugene, Oregon** – City put a moratorium on the use of weed killers containing glyphosate on city properties.
- **Portland, Oregon** – Since 1988, Portland has restricted the use of Roundup to emergency use only. Glyphosate is banned on all city-owned property.

Texas

- **Austin, Texas** – City Council voted to prohibit the spraying of glyphosate on city lands.
- **Denton, Texas** – City Council voted to implement an integrated pest management program and restrict the use of glyphosate on city parks, fields and playgrounds.

Vermont

Multiple bills containing restrictions or bans on glyphosate have been introduced in the legislature.

Representative Mari Cordes introduced H. 301, which would ban the sale, use or application of the herbicide glyphosate.

Representative Annmarie Christensen introduced H. 328, an act relating to the use of glyphosate herbicide.

Virginia

- **Charlottesville, Virginia** – Restricts the use of glyphosate on any city-owned parks, schools, or buildings. Glyphosate can only be sprayed under limited circumstances.

Washington

- **King County, Washington** – Passed municipal ordinance initiating an Integrative Pest Management (IPM) program to determine if and how pesticides should be used.
- **Kitsap County, Washington** – Passed measure banning the spraying of glyphosate by workers on county-owned and maintained properties. Glyphosate may only be used on noxious weeds as a tool of last resort.
- **Olympia, Washington** – City passed a resolution to encourage the implementation of an Integrative Pest Management (IPM) program for non-chemical pest control.
- **Thurston County, Washington** –Passed municipal ordinance to restrict the use of toxic pesticides on public property.

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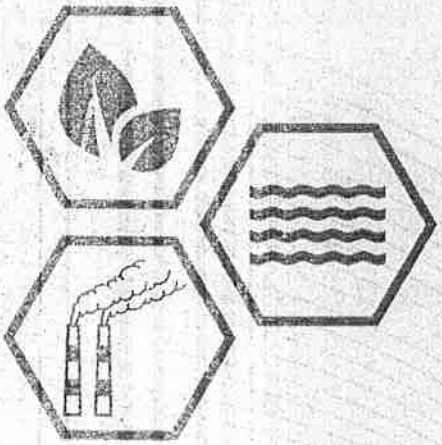

Toxicological Profile for Glyphosate

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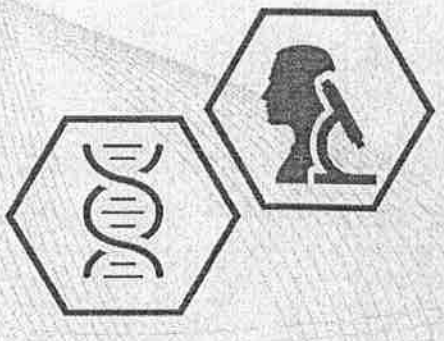

ATSDR

U.S. Department of Health and Human Services
Agency for Toxic Substances and Disease Registry

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FOREWORD

This toxicological profile is prepared in accordance with guidelines developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for these toxic substances described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a relevance to public health discussion which would allow a public health professional to make a real-time determination of whether the presence of a particular substance in the environment poses a potential threat to human health. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to the protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a toxic substance to ascertain the levels of significant human exposure for the substance and the associated acute, intermediate, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine the levels of exposure that present a significant risk to human health due to acute, intermediate, and chronic duration exposures; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public. ATSDR plans to revise these documents in response to public comments and as additional data become available. Therefore, we encourage comments that will make the toxicological profile series of the greatest use.

Electronic comments may be submitted via: www.regulations.gov. Follow the on-line instructions for submitting comments.

Written comments may also be sent to: Agency for Toxic Substances and Disease Registry
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The toxicological profiles are developed under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980, as amended (CERCLA or Superfund). CERCLA section 104(i)(1) directs the Administrator of ATSDR to "...effectuate and implement the health related authorities" of the statute. This includes the preparation of toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL) and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list. In addition, ATSDR has the authority to prepare toxicological profiles for substances not found at sites on the NPL, in an effort to "...establish and maintain inventory of literature, research, and studies on the health effects of toxic substances" under CERCLA Section 104(i)(1)(B), to respond to requests for consultation under section 104(i)(4), and as otherwise necessary to support the site-specific response actions conducted by ATSDR.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staffs of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and is being made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.

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VERSION HISTORY

Date	Description
April 2019	Draft for public comment toxicological profile released

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ATSDR scientists review peer reviewers' comments and determine whether changes will be made to the profile based on comments. The peer reviewers' comments and responses to these comments are part of the administrative record for this compound.

The listing of peer reviewers should not be understood to imply their approval of the profile's final content. The responsibility for the content of this profile lies with ATSDR.

CONTENTS

APPENDICES

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1. RELEVANCE TO PUBLIC HEALTH

1.1 OVERVIEW AND U.S. EXPOSURES

Glyphosate is a phosphonoglycine non-selective herbicide, first registered for use by the EPA in 1974. Glyphosate is typically manufactured for commercial use as a salt available in soluble liquid and granule formulations. Herbicide formulations employing glyphosate salts are commonly produced in combination with additives, inert ingredients, and surfactants. The salt derivatives enhance absorption of glyphosate from the surface of the plant or leaf structure, but are not the herbicidally active portion of the compound. Specific formulations vary in composition and are marketed under numerous trade names (NPIRS 2017; PAN 2009). Commercial products containing glyphosate may have concentrations ranging from 0.96 to 94 w/w%. For example, the common herbicide, Roundup®, has product formulations containing glyphosate in concentrations ranging from 0.96% to as much as 71% (w/w) (NPIRS 2017; PAN 2016b).

Glyphosate is the active ingredient in a variety of broad spectrum herbicidal products for residential, commercial, and agricultural purposes. Selected agricultural commodities such as roundup-ready corn and soybeans have been genetically modified to be resistant to damage when glyphosate is applied to control undesirable weeds. Glyphosate is produced commercially in the United States as a technical-grade substance with a purity of ≥95% (McBean 2011). In 2007, U.S. agricultural use of glyphosate was approximately 82,800 tons and non-agricultural use of glyphosate was approximately 9,300 tons (Battaglin et al. 2014). In 2014, U.S. agricultural use of glyphosate was approximately 124,953 tons and non-agricultural use of glyphosate was approximately 13,260 tons (Benbrook 2016). The manufacture and use of glyphosate has led to its direct release into the environment (EPA 1993). Once glyphosate enters the environment, it has low potential for environmental bioavailability and is unlikely to bioaccumulate; the chemical is either degraded by microbial processes or inactivated by adsorption to soil (Shushkova et al. 2010; Smith and Oehme 1992). Glyphosate is expected to adsorb to soils under most environmental conditions; therefore, leaching into groundwater is minimal (Smith and Oehme 1992). Glyphosate may enter surface waters due to its use in some aquatic environments. Volatilization of glyphosate is not an important fate process based on its low vapor pressure and ionic nature (Smith and Oehme 1992). Transport in the air after spray applications is dependent on meteorological conditions; ground and aerial applications can result in spray drift, which may affect non-target plants (PAN 2009; Yates et al.1978).

The general population may be exposed to glyphosate by dermal contact with consumer products, crops, foliage, or soils containing residues of this chemical; ingestion of plants, crops, foods, or waters containing residues of this chemical; and inhalation of mist or spray during the use of products containing this chemical. As a result of its widespread usage, glyphosate is present at low levels in a wide range of foods (FAO and WHO 2016). The greatest potential for exposure can be expected for people who use glyphosate products at home and for populations residing near agricultural areas and crop farms, manufacturing and processing plants where glyphosate is produced or used, and hazardous waste disposal sites containing glyphosate.

Occupational exposure of glyphosate may occur via inhalation, dermal contact, and/or ocular contact during manufacture, transport, use, and disposal. Farmers and home gardeners using herbicides containing glyphosate may be exposed to glyphosate via inhalation, dermal contact, and/or ocular contact as well. People may be exposed to glyphosate upon entering areas where it has been recently applied. Dermal contact appears to be the major route of exposure to glyphosate for people involved in its application.

Children are expected to be exposed to glyphosate by the same routes as adults in the general population. Products containing glyphosate should be kept out of the reach of children. Due to increased hand-to-mouth activity and playing habits, children are more likely to come into contact with glyphosate residues that may be present in soil. Glyphosate is not likely to bioaccumulate in breast milk (Bus 2015) and was not detected in breast milk from lactating mothers with detectable glyphosate in their urine (McGuire et al. 2016). In one small study, neither glyphosate nor its major degradation product, aminomethylphosphonic acid (AMPA), were detected in the maternal or fetal cord serum of pregnant subjects (Aris and LeBlanc 2011).

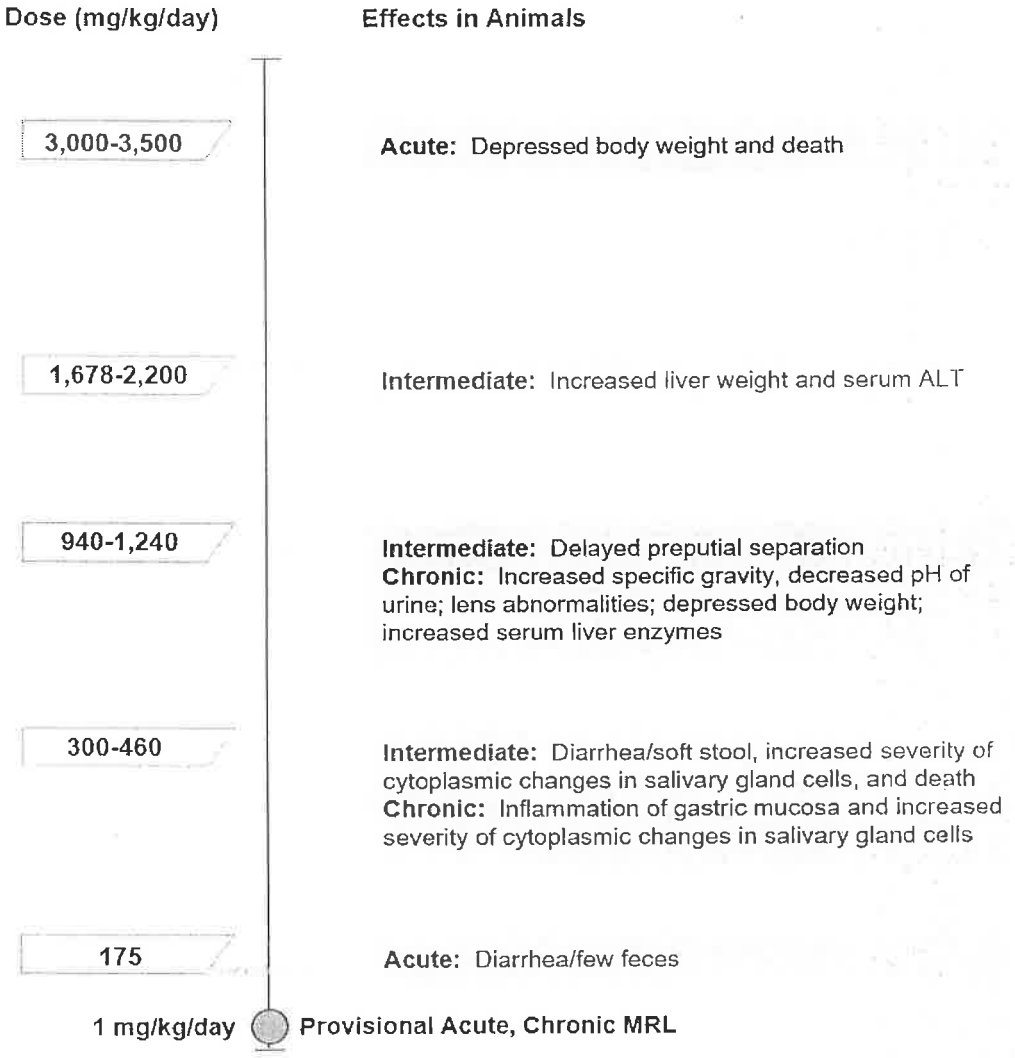
See Chapter 5 for more detailed information regarding concentrations of glyphosate in environmental media.

1.2 SUMMARY OF HEALTH EFFECTS

Information regarding the toxicity of glyphosate comes primarily from oral studies in laboratory animals exposed to glyphosate technical. No information was located regarding health effects in humans exposed to glyphosate technical; human exposures are to herbicides that contain glyphosate and other ingredients or to glyphosate residues in selected food sources. Human studies have reported possible associations

between glyphosate herbicide use and various health outcomes. A few animal studies evaluated the effects of inhalation or oral exposure to glyphosate formulations containing surfactant and additional unspecified substances. Reported effects may be due, at least in part, to the surfactant. Furthermore, glyphosate formulations vary in specific components and their relative proportions, thus precluding meaningful comparisons of toxic effect levels. Therefore, Figure 1-1 contains summary information related only to glyphosate technical.

Figure 1-1. Noncancer Health Effects Found in Animals Following Oral Exposure to Glyphosate Technical



Exposure Durations: Acute (≤14 days); Intermediate (15-364 days); Chronic (≥365 days)

As illustrated in Figure 1-1, gastrointestinal disturbance and effects on the salivary gland appear to be the most sensitive noncancer effects in animal studies that employed oral exposure to glyphosate technical. Ocular, hepatic, renal, and body weight effects have been reported as well. Developmental effects were observed at dose levels resulting in maternal toxicity. Effects observed in animals are considered relevant to human health in the absence of experimental data to indicate otherwise.

Gastrointestinal Effects. Gastrointestinal symptoms (e.g., nausea, vomiting, abdominal pain, sore throat, mucosal damage in mouth and esophagus) are commonly reported in patients ingesting glyphosate products (Chang et al. 1999; Lee et al. 2000, 2008; Moon and Chun 2010; Roberts et al. 2010; Sawada et al. 1988; Talbot et al. 1991; Tominack et al. 1991). Gastrointestinal effects have frequently been seen in animal studies. For example, soft stool/diarrhea were reported in pregnant rabbits gavaged with glyphosate technical during gestation (EPA 1992f, 2017b) and rats administered glyphosate technical in the diet for 2 generations (EPA 1992a). Inflammation of gastric mucosa was observed in female rats orally exposed to glyphosate technical for 2 years (EPA 1991a, 1991b). Cytoplasmic alterations were reported in salivary glands of glyphosate-treated rats and mice; the toxicological significance of these salivary gland changes is uncertain (NTP 1992).

Body Weight Effects. Depressed body weight was observed during intermediate- and chronic-duration oral exposure of laboratory animals to glyphosate technical at doses ≥1,183 mg/kg/day (EPA 1985a, 1991a, 1991b, 1992a).

Hepatic Effects. Increased liver weight and increased serum markers of liver effects (alkaline phosphatase [AP], alanine aminotransferase [ALT], and/or bile acids) were observed in rats administered glyphosate technical for 13 weeks at ≥1,678 mg/kg/day (NTP 1992). Centrilobular hepatocellular necrosis was observed in livers from male mice administered glyphosate technical for 2 years at an estimated dose of 4,945 mg/kg/day (EPA 1985a).

Renal Effects. Increased specific gravity of urine and decreased urinary pH were noted among male rats administered glyphosate technical for 2 years at 940 mg/kg/day (EPA 1991a, 1991b). Female mice administered glyphosate technical for 2 years at 6,069 mg/kg/day exhibited significantly increased incidence of renal proximal tubule epithelial basophilia and hypertrophy (EPA 2015a).

Ocular Effects. In a report of human case series of 1,513 ocular exposures to glyphosate products, minor symptoms (primarily transient irritation) were observed in 70% of the cases; most (99%) complained of

eye pain (Acquavella et al. 1999). Lens abnormalities were observed in male rats administered glyphosate technical for 2 years at 940 mg/kg/day (EPA 1991a, 1991b). According to EPA (1993), glyphosate is considered mildly irritating to the eye following ocular instillation.

Developmental Effects. Limited epidemiology studies provided suggestive evidence of associations between maternal preconception exposure to glyphosate and increased risk of spontaneous abortion (Arbuckle et al. 2001) and parent-reported attention deficit disorder/attention deficit hyperactivity disorder (Garry et al. 2002). Depressed weight and increased incidence of unossified sternebrae were observed in gestation day (GD) 20 fetuses from rat dams treated with glyphosate technical by gavage at 3,500 mg/kg/day during GDs 6–19 (EPA 1992e). In a study of rats exposed via the diet for 2 generations, up to 14–20% depressed pup body weight and/or body weight gain were noted at an estimated glyphosate technical dose of 3,134 mg/kg/day (EPA 1992a). In another 2-generation oral rat study, an estimated glyphosate technical dose of 1,234 mg/kg/day resulted in delayed preputial separation (EPA 2013a).

Cancer Effects. The carcinogenic potential of glyphosate has been evaluated in three meta-analyses (Chang and Delzell 2016; IARC 2017; Schinasi and Leon 2014) and a number of case-control and cohort epidemiology studies (see Section 2.19 for detailed information and specific citations). The meta-analyses reported positive associations between glyphosate use and selected lymphohematopoietic cancers. Most of the case-control and cohort studies used self-reported ever/never glyphosate use as the biomarker of exposure, and subjects were likely exposed to other pesticides as well. Numerous studies reported risk ratios greater than 1 for associations between glyphosate exposure and risk of non-Hodgkin's lymphoma or multiple myeloma; however, the reported associations were statistically significant only in a few studies.

Collectively, animal studies in which glyphosate-containing herbicide formulations were tested by the oral exposure route have identified the following targets of toxicity:

- Body weight effects (depressed body weight gain in mice),
- Hematological effects (decreases in red blood cells, hematocrit, and hemoglobin, and increases in mean corpuscular volume and neutrophils in mice),
- Hepatic effects (increased serum liver enzyme activity and histopathologic liver lesions in male rats),
- Renal effects (histopathologic kidney lesions in male rats), and
- Reproductive effects (increased percentage of morphologically abnormal sperm in rats).

A summary figure of sensitive targets of glyphosate-containing herbicide formulations is not included in this toxicological profile for glyphosate because formulations were not equivalent across studies and other ingredients (in addition to glyphosate as active ingredient) may have influenced the observed effects.

1.3 MINIMAL RISK LEVELS (MRLs)

Animal studies submitted to EPA's Office of Pesticides Programs to fulfill requirements for the registration of a particular glyphosate formulation for use in the United States involve exposure to glyphosate technical (typically <90% purity). Some animal studies in the open literature used glyphosate formulations that typically included 1–41% glyphosate technical (or glyphosate salts) and up to 18% surfactant (along with other "inert" ingredients). Surfactants in glyphosate formulations may be at least partly responsible for the toxic effects from overexposure to glyphosate formulations (Adam et al. 1997; Sawada et al. 1988; Williams et al. 2000). Human exposure to glyphosate formulations via its use in weed control includes exposure to all substances in a particular glyphosate formulation. No MRLs were derived for glyphosate formulations due to the wide variation in glyphosate content and surfactants used in various glyphosate formulations and the fact that surfactants can contribute to the toxicity of glyphosate formulations. However, because exposures of the general population via food or water sources with measurable glyphosate residues most likely involve glyphosate and/or its breakdown products rather than the intact glyphosate-based formulation, health effects data associated with oral exposure to glyphosate technical are considered relevant to potential derivation of oral MRLs for glyphosate. Oral MRLs based on glyphosate technical would not be applicable to intentional or accidental ingestion of a glyphosate formulation.

Available data for inhalation exposure to glyphosate technical are limited to a summary from a single 4-week repeated-exposure rat study in which no effects were observed at the highest exposure level (EPA 1985c). The inhalation database was, therefore, not considered adequate for derivation of provisional inhalation MRLs for glyphosate. As presented in Figure 1-1, available data have identified the gastrointestinal tract as the most sensitive target of glyphosate toxicity following oral exposure. The oral database was considered adequate for derivation of provisional acute- and chronic-duration oral MRLs for glyphosate. These provisional MRLs are summarized in Table 1-1 and discussed in detail in Appendix A. The provisional chronic-duration MRL value is adopted as the provisional intermediate-duration oral MRL for glyphosate (see Appendix A).

As illustrated in Figure 1-2, gastrointestinal disturbances (e.g., loose stools/diarrhea, decreased fecal production, inflammation of gastric mucosa, cytoplasmic alterations in salivary glands) appear to be the most sensitive effects of glyphosate technical toxicity in animals. The lowest-observed-adverse-effect levels (LOAELs) in Figure 1-2 reflect actual doses (levels of exposure) employed in animal studies.

Figure 1-2. Summary of Sensitive Targets of Glyphosate Technical – Oral

The gastrointestinal tract is the most sensitive target of ingested glyphosate technical. Numbers in circles are the lowest LOAELs for all health effects in animals; no reliable dose-response data were available for humans.

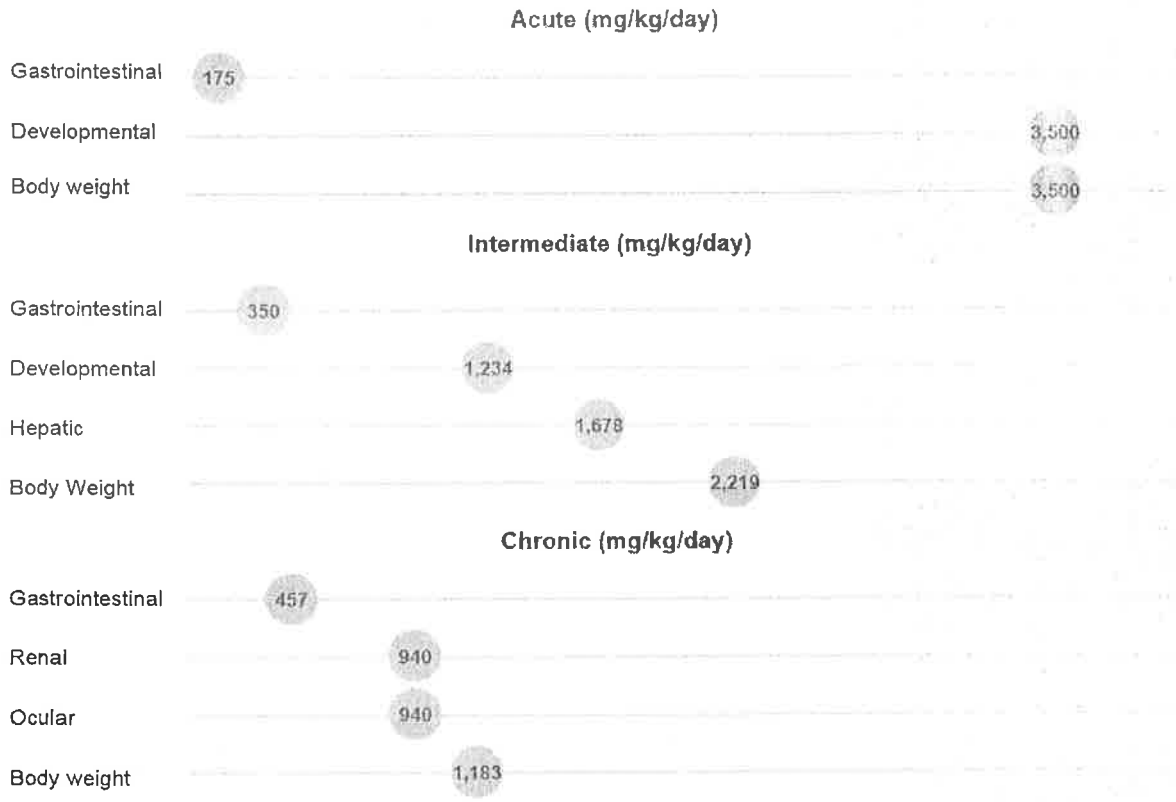


Table 1-1. Minimal Risk Levels (MRLs) for Glyphosate[a]

Exposure duration	Provisional MRL	Critical effect	Point of departure	Uncertainty factor	Reference
Inhalation exposure (ppm)					
Acute	Insufficient data for MRL derivation				
Intermediate	Insufficient data for MRL derivation				
Chronic	Insufficient data for MRL derivation				
Oral exposure (mg/kg/day)					
Acute	1	Gastrointestinal effects	100 (NOAEL)	100	EPA 2017b
Intermediate	The provisional chronic-duration oral MRL of 1 mg/kg/day is adopted as the provisional intermediate-duration oral MRL.				
Chronic	1	Gastrointestinal effects	113 (NOAEL)	100	EPA 1991a, 1991b

[a]See Appendix A for additional information.

LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

CHAPTER 2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of glyphosate. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health. When available, mechanisms of action are discussed along with the health effects data; toxicokinetic mechanistic data are discussed in Section 3.1.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, as well as people exposed during production and/or use of glyphosate-containing products, the information in this section is organized by health effect. These data are discussed in terms of route of exposure (inhalation, oral, and dermal) and three exposure periods: acute (≤14 days), intermediate (15–364 days), and chronic (≥365 days).

As discussed in Appendix B, a literature search was conducted to identify relevant studies examining health effect endpoints. Figure 2-1 for glyphosate technical and Figure 2-2 for glyphosate formulations provide an overview of the database of studies in humans or experimental animals included in this chapter of the profile. These studies evaluate the potential health effects associated with inhalation, oral, or dermal exposure to glyphosate, but may not be inclusive of the entire body of literature.

This ATSDR Toxicological Profile for Glyphosate includes data for glyphosate technical (purity typically >95%) and glyphosate formulations (typically 1–41% v/v glyphosate technical or glyphosate salts and ≤18% polyoxyethyleneamine [POEA] surfactant). Surfactants in glyphosate formulations may be at least partly responsible for the toxic effects from exposure to glyphosate formulations (Adam et al. 1997; Sawada et al. 1988; Williams et al. 2000). As such, health effects observed in studies of animals exposed to relatively high levels of glyphosate technical may not accurately reflect health effects from human exposure to glyphosate formulations during application as an herbicide. However, because the general population may be exposed to glyphosate and/or its breakdown products (rather than to a particular glyphosate formulation) in selected food sources or contaminated drinking water, health effects from

animal studies in which glyphosate technical was used as test substance are considered relevant to human health.

Product names and reported descriptions for glyphosate-containing products included in this toxicological profile are summarized in Table 2-1 by reference (alphabetical order). Hereafter, each glyphosate-containing formulation will generally be identified only by the reported product name.

Table 2-1. Description of Selected Glyphosate Formulations

Reference	Product name	Product description[a]
Adam et al. 1997	Roundup®	41% w/v glyphosate isopropylamine salt and 18% w/v POEA
Benedetti et al. 2004	Glyphosate-Biocarb®	360 g/L glyphosate and 18% w/v POEA
Bolognesi et al. 1997	Roundup®	30.4% glyphosate
Caglar and Kolankaya 2008	Roundup®	Monsanto of Brazil; 360 g/L glyphosate, 18% w/v POEA
Cassault-Meyer et al. 2014	Roundup® Grand Travaux Plus	607 g/L glyphosate isopropylamine salt and adjuvants such as POEA
Contardo-Jara et al. 2009	Roundup Ultra®	360 g/L glyphosate isopropylamine salt and surfactants of unspecified composition
Dallegrave et al. 2003, 2007	Roundup®	Monsanto of Brazil; 360 g/L glyphosate, 18% w/v POEA
Dimitrov et al. 2006	Roundup®	Ingredients and proportions not specified
EPA 1985c	Roundup®	33.3% use dilution (41.56% isopropylamine salt of glyphosate in concentrate)
Feng et al. 1990a	Roundup®	Unspecified proportion of glyphosate isopropylamine salt
Gasnier et al. 2009	Roundup Grands Travaux®	40% glyphosate
George et al. 2010	Roundup Original®	41% glyphosate and 15% POEA
Grisolia 2002	Roundup®	48% glyphosate isopropylammonium salt; 12% inerts, including POEA
Holečková 2006	Unspecified technical herbicide	62% w/w isopropylamine salt of glyphosate and 38% unspecified inerts
Jasper et al. 2012	Roundup Original®	41% glyphosate and 16% POEA
Kale et al. 1995	Roundup®	Glyphosate isopropylamine salt of unspecified concentration
Koller et al. 2012	Roundup Ultra Max®	450 g/L glyphosate acid
Maibach 1986	Roundup®	41% glyphosate as isopropylamine salt, water, surfactant
Mao et al. 2018	Roundup®	Composition not specified
Moriya et al. 1983	Roundup®	Composition not specified

Table 2-1. Description of Selected Glyphosate Formulations

Reference	Product name	Product description[a]
Panzacchi et al. 2018	Roundup Bioflow®	41.5% glyphosate isopropylamine salt, 42.5% water, and 15% proprietary surfactant
Paz-y-Miño et al. 2007	Roundup-Ultra®	Unspecified proportions of glyphosate, POEA, and the adjuvant Cosmoflux 411F
Peluso et al. 1998	Roundup®	30.4% glyphosate isopropylammonium salt
Piešová 2004, 2005	Unspecified product from Monsanto, Antwerp, Belgium	62% w/w isopropylamine salt of glyphosate and 38% unspecified inerts
Prasad et al. 2009	Roundup®	>41% glyphosate isopropylamine salt
Raipulis et al. 2009	Roundup BIO®	Ingredients not specified
Ramos-Morales et al. 2008	Roundup®	Not specified
Rank et al. 1993	Roundup®	480 g/L glyphosate isopropylamine salt
Rodrigues et al. 2011	Roundup®	Not specified
Romano et al. 2010	Roundup Transorb®	648 g/L isopropylamine salt of glyphosate and 594 g/L inerts
Šiviková and Dianovskỳ 2006	Unspecified product from Monsanto Europe S.A., Belgium	62% glyphosate; 38% unspecified inerts
Vigfusson and Vyse 1980	Roundup®	Ingredients not specified
Wester et al. 1991	Roundup®	Ingredients not specified
Wildeman and Nazar 1982	Unspecified commercial formulation	Glyphosate-containing product (no additional details on composition)
Wunnapuk et al. 2014	Concentrate Roundup® Weedkiller	Monsanto Australia, containing 360 g/L of glyphosate (only ingredient specified)

[a]Limited to the glyphosate-containing substance description in the corresponding study report.

POEA = polyoxyethyleneamine (surfactant)

Animal oral study information for glyphosate technical is presented in Table 2-2 and Figure 2-3. Animal oral study information for glyphosate formulations is presented in Table 2-3. Animal dermal study information for glyphosate technical is presented in Table 2-4.

Levels of significant exposure (LSEs) for each route and duration are presented in tables and illustrated in figures. LSE tables and figures for animal inhalation studies of glyphosate technical and glyphosate formulations are precluded by lack of data. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality

(e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an endpoint should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these endpoints. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

A User's Guide has been provided at the end of this profile (see Appendix C). This guide should aid in the interpretation of the tables and figures for LSEs and MRLs.

Glyphosate-containing products are among the most widely-used herbicides in commercial, agricultural, and residential settings (NPIC 2015). Selected field crops have been genetically modified to resist damage from glyphosate; such crops can be sprayed with glyphosate formulations to control weed growth without harming the genetically-modified plants. Selected glyphosate-containing products are labeled for use as desiccants on some grain crops a few weeks prior to harvest.

Glyphosate technical (purity typically >95%) has been evaluated in numerous animal studies, most of which employed the oral exposure route and were submitted to EPA's Office of Pesticide Programs through the pesticide registration program as directed by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Federal Food, Drug and Cosmetic Act (FFDCA), and Food Quality Protection Act (FQPA). The submitted studies are generally unpublished proprietary studies. EPA evaluated submitted study reports and produced summaries termed Data Evaluation Records or Data Evaluation Reports (DERs) that include study details and EPA's own conclusions regarding study design, results, and conclusions of the study authors. Information from DERs received from EPA is summarized in this ATSDR Toxicological Profile for Glyphosate (note: selected DERs can be requested at: https://www.epa.gov/foia or viewed from a list of cleared reviews for glyphosate or glyphosate salts at https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/html/a.html). EPA evaluated and produced DERs for selected proprietary animal studies submitted by various chemical companies to

agencies or organizations outside the United States for product registration purposes. Results from the DERs available to ATSDR were included in the Toxicological Profile for Glyphosate.

Epidemiological studies of glyphosate are predominantly case-control and cohort studies that examined possible associations between exposure to glyphosate (in glyphosate-containing herbicides) and selected health outcomes (noncancer and cancer endpoints), or case reports following accidental or intentional ingestion of glyphosate-containing products. These epidemiology studies are summarized in Table 2-5 (noncancer) and Table 2-7 (cancer). The majority of the studies used self-reported (or proxy reported) ever/never glyphosate use as the measure of exposure and some studies included a metric for frequency of exposure. There is no information regarding health effects in humans exposed to glyphosate technical.

Most reliable dose-response health effects data come from oral studies of animals administered glyphosate technical (see Figure 2-1 for an overview of the number of animal studies examining potential endpoints of concern from oral exposure to glyphosate technical). No information was located regarding the effects of inhaled glyphosate technical. In a 4-week study that employed repeated inhalation exposure of rats to Roundup®, no adverse effects were observed at the highest exposure concentration tested (360 mg Roundup®/m^3) (EPA 1985c). Limited animal data for dermal exposure to glyphosate technical indicate that glyphosate is not a dermal irritant. Results from the oral animal studies identify the following targets of glyphosate toxicity, albeit at relatively high dose levels:

- **Gastrointestinal effects:** Clinical signs and/or pathological evidence of glyphosate-induced irritation were observed in several animal studies; the lowest dose level resulting in gastrointestinal effects was 175 mg/kg/day for diarrhea and few feces in pregnant rabbits administered glyphosate acid by gavage. Gastrointestinal disturbances are signs and/or symptoms following ingestion of large amounts of glyphosate-containing products.

- **Developmental effects:** Glyphosate treatment-related developmental effects were noted in a few studies at dose levels (≥1,234 mg/kg/day) resulting in maternal toxicity as well.

- **Body weight effects:** Depressed body weight and/or depressed body weight gain resulted from repeated dosing of glyphosate technical at dose levels ≥1,183 mg/kg/day.

- **Hepatic effects:** Increases in liver weight and serum ALT activity were observed in one repeated-dose study at a dose level of 1,678 mg/kg/day.

- **Ocular effects:** Lens abnormalities were observed in one repeated-dose study at a dose level of 940 mg/kg/day.

- **Renal effects:** Indicators of renal toxicity were noted in rats and mice administered glyphosate technical in the diet for 2 years at high doses (940 and 6,069 mg/kg/day, respectively).

- **Other effects:** Neurological, hematological, immunological, and reproductive endpoints have been evaluated, but do not appear to be particular targets of glyphosate toxicity.

- **Cancer:** Upon evaluation of available carcinogenicity studies in laboratory rodents, a number of agencies or organizations have concluded that glyphosate technical does not appear to be an animal carcinogen. In contrast, IARC considered the animal data to provide "*sufficient evidence*" of glyphosate carcinogenicity.

An overview of the number of human and animal studies examining potential endpoints of concern from exposure to glyphosate formulations is presented in Figure 2-2. Results from available animal studies identify the following targets of toxicity:

- **Developmental effects:** Histopathologic testicular lesions, decreased sperm production, and increased incidence of fetal skeletal malformations were reported in response to oral dosing of rat weanlings or pregnant rats with selected glyphosate formulations in the range of 5–500 mg/kg/day.

- **Endocrine effects:** Decreased serum testosterone was noted in male rat weanlings administered a glyphosate formulation orally at 5 mg/kg/day.

- **Body weight effects:** Seriously depressed body weight gain was observed in mice administered a glyphosate formulation orally at 50 mg/kg/day.

- **Renal effects:** Histopathologic kidney lesions were noted in male rats gavaged once with a glyphosate formulation at 250 mg/kg.

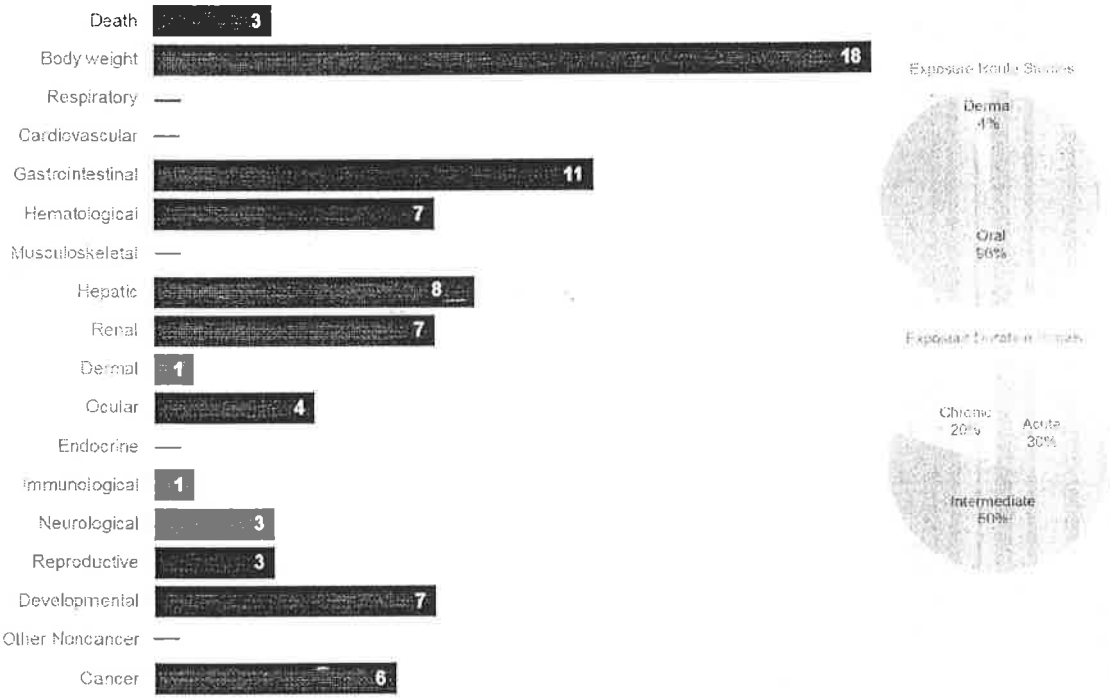
- **Hepatic effects:** Increased serum liver enzyme activity and histopathologic liver lesions were reported in male rats repeatedly gavaged with a glyphosate formulation at 487 mg/kg/day.

- **Hematological effects:** Decreases in red blood cells, hematocrit, and hemoglobin, and increases in mean corpuscular volume and neutrophils were reported in mice administered a glyphosate formulation orally at 500 mg/kg/day.

- **Reproductive effects:** Increased percentage of morphologically abnormal sperm was reported among rats receiving a glyphosate formulation from the drinking water for 8 days at 640 mg/kg/day.

Figure 2-1. Overview of the Number of Animal Studies Examining Glyphosate Technical Health Effects*

Most studies examined the potential body weight, gastrointestinal, hematological, hepatic, and developmental effects of glyphosate technical (counts represent studies examining endpoint)

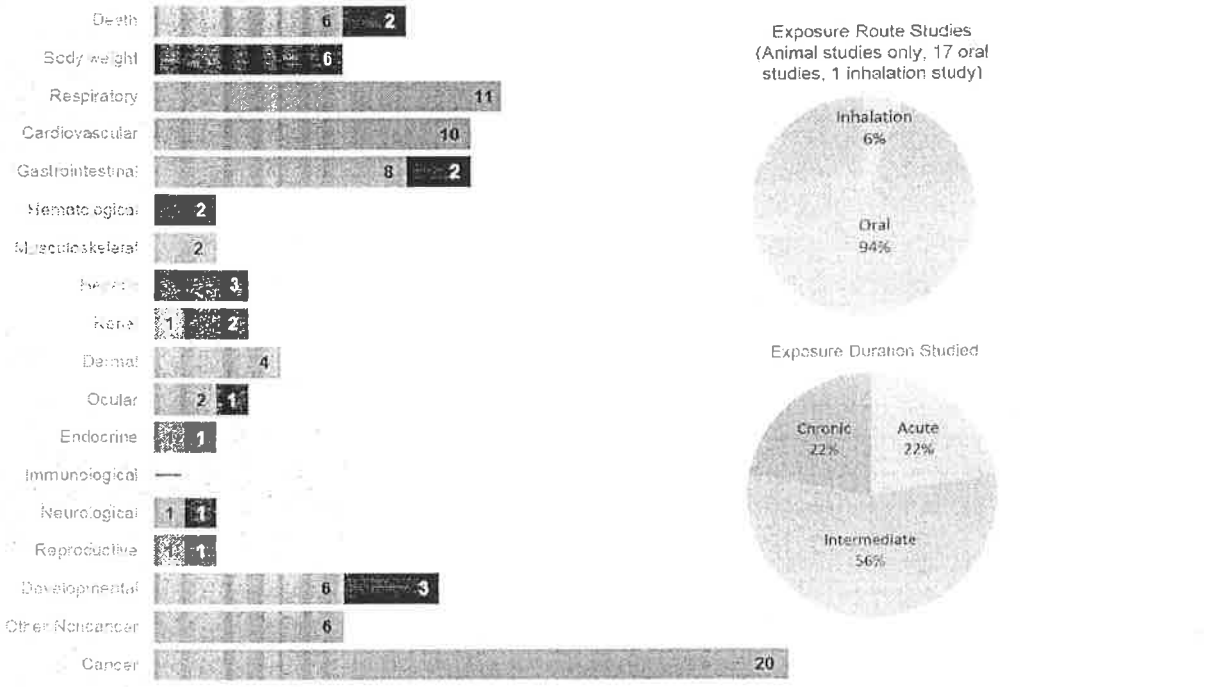


*Includes only animal studies that employed oral exposure to glyphosate technical as discussed in Chapter 2. A total of 22 studies include those finding no effect. Most studies examined multiple endpoints.

Figure 2-2. Overview of the Number of Studies Examining Glyphosate Formulations Health Effects*

Most epidemiological studies examined potential cancer, respiratory, and developmental effects associated with glyphosate-containing products; most animal studies examined potential body weight and developmental effects associated with glyphosate-containing products

More studies evaluated health effects in humans than animals (counts represent studies examining endpoint)



*A total of 42 studies, including those finding no effect. Many studies examined multiple endpoints. Reliable exposure route and duration information was not typically available for humans. Therefore, relative exposure route and duration proportions are plotted only for animal studies.

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE (≤14 days)									
1	Rat (Wistar) 8 M	Once (G)	0, 2,000	CS, GN, HP, LE, OW	Gastro		2,000		Diarrhea in 2/8 rats for 6 hours postdosing, resolving by sacrifice at 24 hours
Adam et al. 1997 – Glyphosate technical, purity not specified									
2	Rat (Sprague-Dawley) 5 (mixed)	Once (GW)	3,160, 3,980, 5,010, 6,310	CS, GN, LE	Death			4,320	LD_{50}
EPA 1992b – Glyphosate technical, purity not specified									
3	Rat (Sprague-Dawley) 25 F	GDs 6–19 1 time/day (GW)	0, 300, 1,000, 3,500	BW, CS, DX, FX, GN, LE, MX, TG	Death			3,500	6/25 Dams died
					Bd Wt	1,000		3,500	28.5% depressed mean maternal body weight gain
					Gastro	1,000		3,500	Diarrhea, soft stools
					Develop	1,000	3,500		9% depressed mean fetal body weight, increased incidence of unossified sternebrae at serious maternally-toxic dose level
EPA 1992e – Glyphosate technical, purity 98.7%									
4	Rat (Alpk: APfSD) 10 M, 10 F	Once (GW)	0, 500, 1,000, 2,000	BW, CS, FI, GN, HP, LE, OF, OW	Bd Wt	2,000			
					Gastro	1,000	2,000		Diarrhea
					Neuro	1,000	2,000		Decreased activity, subdued behavior, hunched posture
					Other	1,000	2,000		Hypothermia
EPA 2013c – Glyphosate technical, purity 95.6%									

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
5	Rat (Alpk: APfSD) 24 F	GDs 7–16 1 time/day (GW)	0, 250, 500, 1,000	BW, CS, DX, FI, FX, GN, LE, MX, OW	Bd Wt Develop	1,000 1,000			
EPA 2017b – Glyphosate acid, purity 95.6%									
6	Rabbit (New Zealand white) 20 F	GDs 8–20 1 time/day (GW)	0, 100, 175, 300	BW, CS, DX, FI, FX, GN, LE, MX, OW	Bd Wt Gastro Develop	300 100[b] 300	175		NOAEL for maternal body weight Diarrhea, few feces
EPA 2017b – Glyphosate acid, purity 95.6%									
INTERMEDIATE EXPOSURE (15–364 days)									
7	Rat (Sprague-Dawley) 30 M, 30 F	2-Generation, up to 19 weeks/ generation (F)	F0 M: 0, 137, 754, 2,219 F0 F: 0, 160, 802, 3,134 F1 M: 0, 165, 818, 2,633 F1 F: 0, 194, 947, 3,035	NS	Bd Wt Gastro Repro Develop	754 M 802 F 754 M 802 F 2,219 M 3,134 F 802	2,219 M 3,134 F 2,219 M 3,134 F	 3,134	Up to 12% depressed mean paternal body weight gain Up to 18% depressed mean maternal body weight gain Soft stool Soft stool Up to 14–20% depressed mean pup body weight or body weight gain during lactation at maternally-toxic dose level
EPA 1992a -- Glyphosate technical, purity 97.67%									
8	Rat (Sprague-Dawley) 12 M, 24 F	3-Generation (F)	0, 3, 10, 30	BW, CS, DX, FI, FX, GN, HP, LE, MX, OW	Bd Wt Repro	30 30			
EPA 1992g -- Glyphosate technical, purity 98.7%									

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
9	Rat (Sprague-Dawley) 28 M, 28 F	2-Generation, up to 19 weeks/ generation (F)	M: 0, 121, 408, 1,234; F: 0, 126, 423, 1,273	BW, CS, DX, FI, FX, GN, HP, LE, MX, OF, OW, TG	Bd Wt	1,234 M 1,273 F			
					Hepatic	1,234 M 1,273 F			
					Renal	1,234 M 1,273 F			
					Repro	1,234 M 1,273 F			
					Develop	408 M	1,234 M		Delayed preputial separation
EPA 2013a – Glyphosate technical, purity 95.7%									
10	Rat (Alpk: APfSD) 12 M, 12 F	13 weeks (F)	M: 0, 155.5, 617.1, 1,546.5 F: 0, 166.3, 672.1, 1,630.6	BW, CS, FI, GN, HP, LE, OF, OW	Neuro	1,546.5 M 1,630.6 F			
EPA 2013c – Glyphosate technical, purity 95.6%									

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
11	Rat (F344/N) 10 M, 10 F	13 weeks (F)	M: 0, 205, 410, 811, 1,678, 3,393 F: 0, 213, 421, 844, 1,690, 3,393	BC, BW, CS, EA, FI, GN, HE, HP, LE, OF, OW	Bd Wt	1,678 M	3,393 M		18% lower mean body weight and body weight gain
						3,393 F			
					Gastro	205	410 M		Increased severity of basophilia and hypertrophy of acinar cells in parotid and submandibular salivary glands
						213	421 F		Increased severity of basophilia and hypertrophy of acinar cells in parotid and submandibular salivary glands
					Hemato	3,393			
					Hepatic	811 M	1,678 M		Increases in liver weight and serum ALT
						1,690 F	3,393 F		Increases in liver weight and serum AP, ALT, and bile acids
NTP 1992 – Glyphosate technical, purity 99%									
12	Mouse (B6C3F1/ Crl) 10 F	28 days (F)	0, 150.1, 449.1, 1,447.5	BW, CS, FI, GN, OF, OW, WI	Bd Wt	1,447.5			
					Immuno	1,447.5			
EPA 2013b – Glyphosate technical, purity 85.2%									

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
13	Mouse (B6C3F1) 10 M, 10 F	13 weeks (F)	M: 0, 507, 1,065, 2,273, 4,776, 10,780 F: 0, 753, 1,411, 2,707, 5,846, 11,977	BW, CS, FI, GN, HP, LE, OF, OW	Bd Wt	2,273 M 5,846 F	4,776 M 11,977 F		11% lower mean final body weight 10% lower mean final body weight
					Gastro	1,065 M 1,411 F	2,273 M 2,707 F		Increased severity of basophilia of acinar cells in parotid salivary gland
					Hepatic	10,780 M 11,977 F			
NTP 1992 – Glyphosate technical, purity 99%									
14	Rabbit (Dutch belted) 16 F	GDs 6–27 1 time/day (GW)	0, 75, 175, 350	BW, CS, DX, FX, GN, LE, MX, TG	Death			350	10/16 maternal rabbits died
					Bd Wt	350			
					Gastro	175	350		Increased incidence of soft stool and/or diarrhea
					Develop	350			
EPA 1992f – Glyphosate technical, purity 98.7%									
CHRONIC EXPOSURE (≥365 days)									
15	Rat (Sprague-Dawley) 60 M, 60 F	Up to 24 months (F)	M: 0, 89, 362, 940 F: 0, 113, 457, 1,183	BC, BW, CS, FI, GN, HE, HP, LE, OW	Bd Wt	940 M 457 F	1,183 F		13% lower mean body weight at treatment week 81
					Gastro	940 M 113 F[c]	457 F		Inflammation of gastric squamous mucosa
					Hemato	940 M 1,183 F			
					Hepatic	940 M 1,183 F			

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
					Renal	362 M	940 M		Increased specific gravity and decreased pH of urine
						1,183 F			
					Ocular	362 M	940 M		Increased incidence of lens abnormalities
						1,183 F			
EPA 1991a, 1991b – Glyphosate technical, purity 96.5%									
16	Rat (Sprague-Dawley) 50 M, 50 F	26 months (F)	M: 0, 3.05, 10.30, 31.45 F: 0, 3.37, 11.22, 34.02	BC, BW, CS, FI, GN, HE, HP, LE, OF, OW, UR	Bd Wt	31.45 M			
						34.02 F			
					Gastro	31.45 M			
						34.02 F			
					Hemato	31.45 M			
						34.02 F			
					Hepatic	31.45 M			
						34.02 F			
					Renal	31.45 M			
						34.02 F			
EPA 1992d – Glyphosate technical, purity 98.7%									

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
17	Rat (Alpk: APfSD Wistar) 64 M, 64 F	Up to 2 years (F)	M: 0, 121, 361, 1,214 F: 0, 145, 437, 1,498	BC, BH, BW, CS, EA, FI, GN, HE, HP, LE, OF, OP, OW, UR	Bd Wt	1,214 M			
						1,498 F			
					Gastro	361 M	1,214 M		Exocrine hyperplasia in pancreas in males
						1,498 F			
					Hemato	1,214 M			
						1,498 F			
					Hepatic	361 M	1,214 M		Increased serum AP, ALT, bilirubin
						437 F	1,498 F		Increased serum AP and ALT
					Renal	361 M	1,214 M		Papillary necrosis in kidney; decreased pH of urine
						437 F	1,498 F		Papillary necrosis in kidney
					Ocular	1,214 M			
						1,498 F			
					Neuro	1,214 M			
						1,498 F			

EPA 2015c – Glyphosate technical, purity 97.6%

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
18	Rat (Sprague-Dawley) 85 M, 85 F	Up to 2 years (F)	0, 10, 100, 300, 1,000	BC, BW, CS, EA, FI, GN, HE, HP, LE, OF, OP, OW, UR	Bd Wt	300	1,000		11–14% lower mean body weight and body weight gain
					Gastro	100	300		Increased severity of basophilia and hypertrophy of acinar cells in parotid and mandibular salivary glands
					Hemato	1,000			
					Hepatic	1,000			
					Renal	300 M	1,000 M		Decreased pH of urine
						1,000 F			
					Ocular	1,000			
EPA 2015c - Glyphosate technical, purity 98.7 and 98.9%									
19	Mouse (CD-1) 50 M, 50 F	24 months (F)	M: 0, 161, 835, 4,945 F: 0, 195, 968, 6,069	BW, CS, FI, GN, HE, HP, LE	Bd Wt	4,945 M			
						6,069 F			
					Gastro	4,945 M			
						6,069 F			
					Hemato	4,945 M			
						6,069 F			
					Hepatic	835 M		4,945 M	Centrilobular hepatocellular necrosis
						6,069 F			
					Renal	4,945 M			
						968 F	6,069 F		Renal tubular epithelial basophilia
EPA 2015a – Glyphosate technical, purity 99.7%									

Table 2-2. Levels of Significant Exposure to Glyphosate Technical – Oral

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
20	Mouse (CD-1) 50 M, 50 F	104 weeks (F)	0, 100, 300, 1,000	BW, CS, FI, GN, HE, HP, LE, WI	Bd Wt	1,000			
					Hepatic	1,000			
					Renal	1,000			
EPA 2015c – Glyphosate technical, purity ≥97.5%									
21	Dog (Beagle) 6 M, 6 F	1 year (C)	0, 20, 100, 500	BC, BW, CS, FI, GN, HE, HP, LE, OP, OW, UR, WI	Bd Wt	500			
					Hemato	500			
					Ocular	500			
EPA 1986a, 1987 – Glyphosate technical, purity 96.13%									

[a]The number corresponds to entries in Figure 2-3; differences in levels of health effects and cancer effects between male and females are not indicated in Figure 2-3. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[b]Used to derive a provisional acute-duration oral MRL for glyphosate; NOAEL divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability); see Appendix A for more detailed information regarding the provisional MRL.
[c]Used to derive a provisional chronic-duration oral MRL for glyphosate; NOAEL divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability); see Appendix A for more detailed information regarding the provisional MRL.

ALT = alanine aminotransferase; AP = alkaline phosphatase; BC = biochemistry; BW or Bd Wt = body weight; C = capsule; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; (F) = exposure in feed; F = female(s); FI = food intake; FX = fetal toxicity; G = gavage, neat; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GW = gavage in water vehicle; HE = hematology; Hemato = hematological; HP = histopathology; Immuno = immunological; LD_{50} = lethal dose, 50% kill; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MRL = Minimal Risk Level; MX = maternal toxicity; NOAEL = no observed-adverse-effect level; NS = not specified; OF = organ function; OP = ophthalmology; OW = organ weight; Repro = reproductive; TG = teratogenicity; UR = urinalysis; WI = water intake

Figure 2-3. Levels of Significant Exposure to Glyphosate Technical – Oral
Acute (≤14 days)

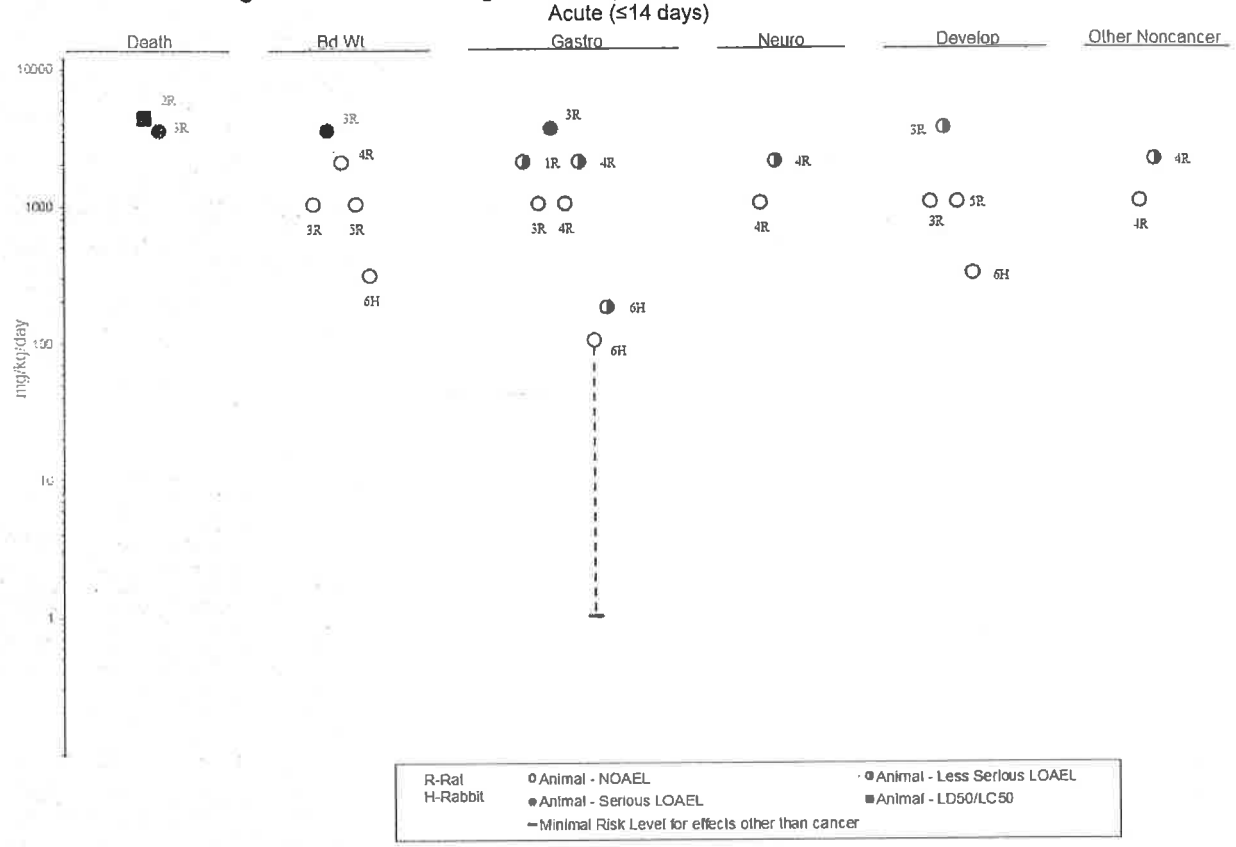


Figure 2-3. Levels of Significant Exposure to Glyphosate Technical – Oral
Intermediate (15-364 days)

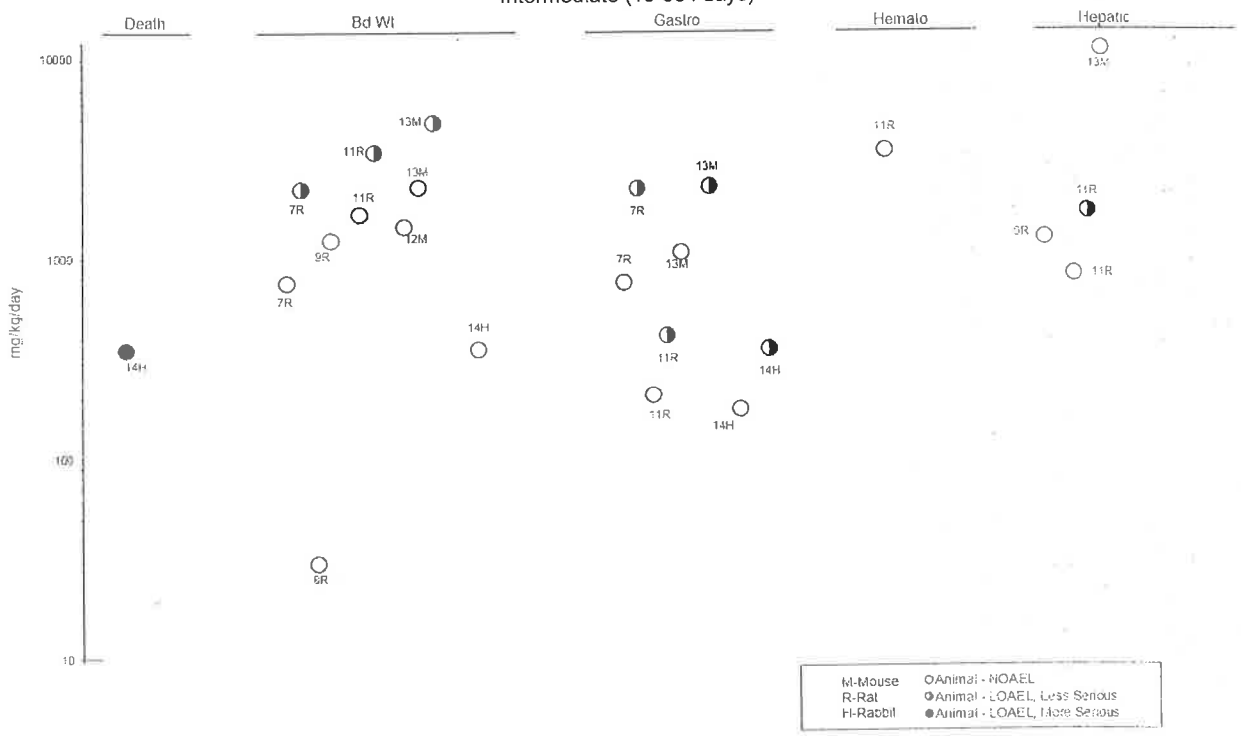


Figure 2-3. Levels of Significant Exposure to Glyphosate Technical – Oral
Intermediate (15-364 days)

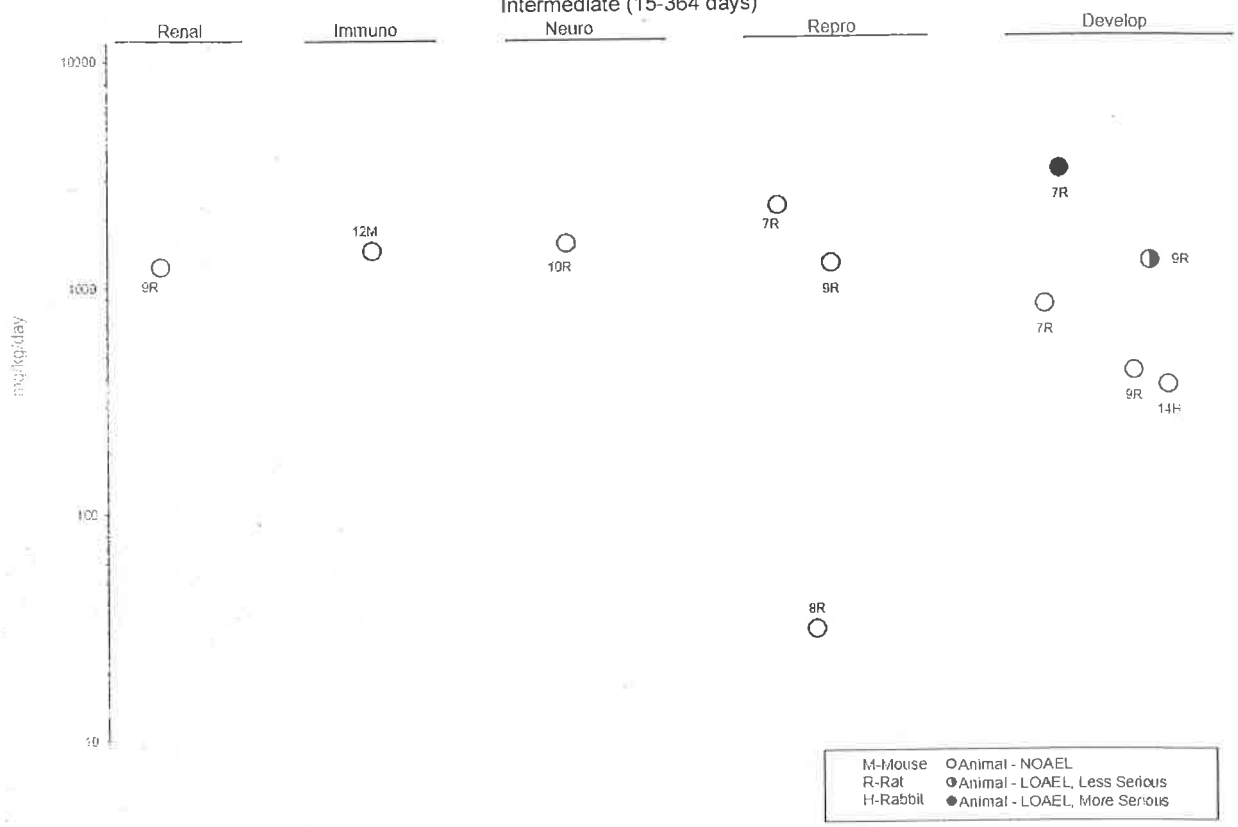


Figure 2-3. Levels of Significant Exposure to Glyphosate Technical -- Oral
Chronic (≥365 days)

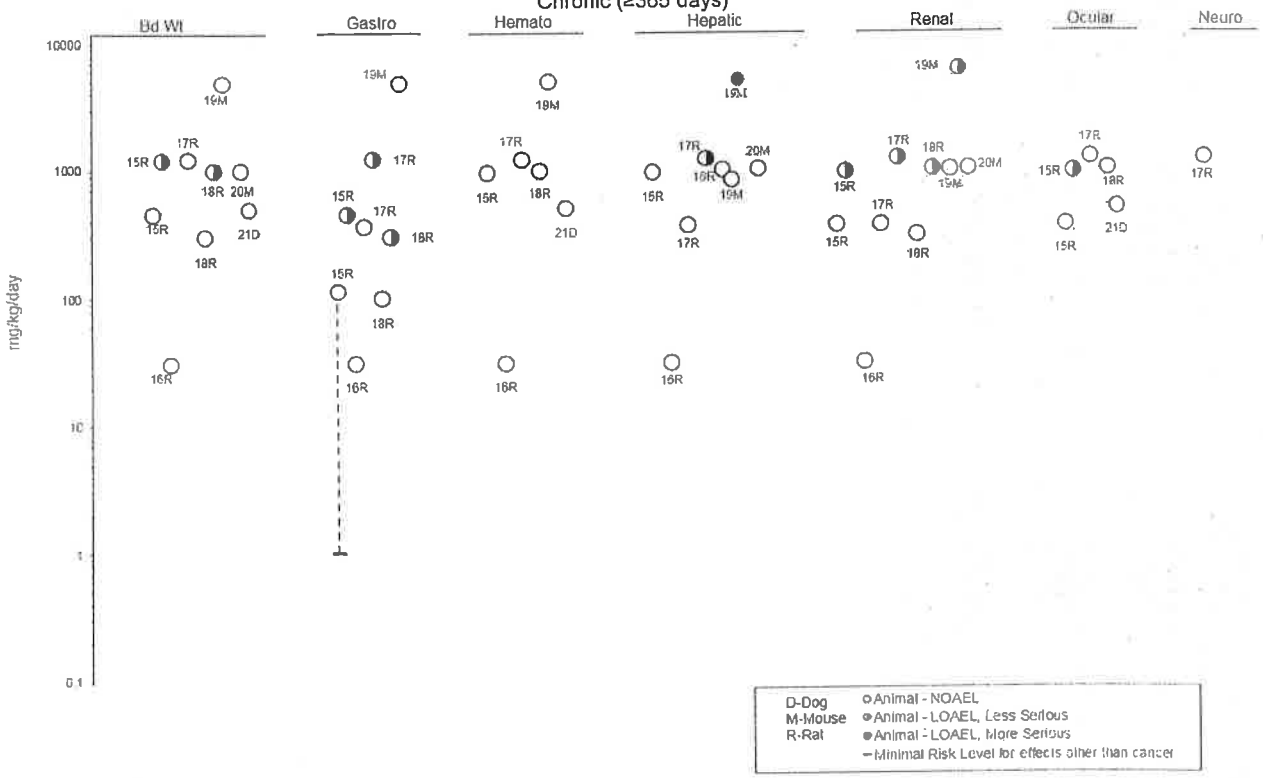


Table 2-3. Levels of Significant Exposure to Glyphosate Formulations – Oral

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
ACUTE EXPOSURE								
Rat (Wistar) 8 M	Once (G)	0, 2,000	CS, GN, HP, LE, OW	Gastro			2,000	Diarrhea in rats administered Roundup® (41% w/v glyphosate isopropylamine salt and 18% w/v polyoxyethyleneamine [POEA]) or glyphosate isopropylamine salt + POEA at the same concentrations as contained in the Roundup® formulation
Adam et al. 1997 – Roundup® (41% w/v glyphosate isopropylamine salt and 18% POEA								
Rat (Sprague-Dawley) 15 M	8 days (W)	0, 640	BW, OF, OW, WI	Repro		640		Up to 18% increased percent abnormal sperm morphology;
Cassault-Meyer et al. 2014 – Roundup® Grand Travaux Plus (607 g/L glyphosate isopropylamine salt and adjuvants such as POEA)								
Rat (Wistar) 15 F	GDs 6–15, 1 time/day (GW)	0, 500, 750, 1,000	BW, DX, FI, FX, GN, HP, LE, MX, OW, TG, WI	Death			1,000 F	8/15 dams died
				Bd Wt	1,000 F			
				Develop		500		Increased incidence of fetal skeletal malformations
Dallegrave et al. 2003 – Roundup® (Monsanto of Brazil; 360 g/L glyphosate, 18% w/v POEA).								
Rat (Wistar) 4 M	Once (GW)	0, 250, 500, 1,200, 2,500	HP, OF	Renal		250 M		Histopathologic kidney lesions.
Wunnapuk et al. 2014 – Concentrate Roundup® Weedkiller (Monsanto Australia, containing 360 g/L of glyphosate)								
INTERMEDIATE EXPOSURE								
Rat (Wistar) 14 or 16 M	75 days, 1 time/ 2 days (GW)	0, 4.87, 48.7, 487	EA, OF	Hepatic	48.7 M	487 M		Increased serum liver enzyme activity, histopathologic liver lesions
Benedetti et al. 2004 – Glyphosate-Biocarb® (360 g/L glyphosate and 18% w/v POEA)								

Table 2-3. Levels of Significant Exposure to Glyphosate Formulations – Oral

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
Rat (Wistar) NS	5 weeks, 1 time/day (GW)	0, 56, 560	BW, EA, FI, HE, HP, OF, OW, WI	Bd Wt	560			
				Hepatic	560			
Caglar and Kolankaya 2008 – Roundup® (Monsanto of Brazil; 360 g/L glyphosate and 18% w/v POEA)								
Rat (Wistar) NS	13 weeks, 1 time/day (GW)	0, 56, 560	BW, EA, FI, HE, HP, OF, OW, WI	Bd Wt	560			
				Hepatic	560			
Caglar and Kolankaya 2008 – Roundup® (Monsanto of Brazil; 360 g/L glyphosate and 18% w/v POEA)								
Rat (Wistar) 15 F	42–44 days (gestation, lactation) (GW)	0, 50, 150, 450	BW, CS, DX, FX, HP, LE, MX, OW, TG	Bd Wt	450 F			
				Develop			50 M	Decreased sperm production, histopathologic testicular lesions
Dallegrave et al. 2007 – Roundup® (Monsanto of Brazil; 360 g/L glyphosate and 18% w/v POEA)								
Mouse (albino Swiss) 10 M, 10 F	15 days 1 time/day (GW)	0, 50, 500	BW, EA, HE, HP, OF	Bd Wt			50	60–66% depressed mean body weight gain
				Hemato	50		500	Decreased red blood cells, hematocrit, hemoglobin; increased mean corpuscular volume, neutrophils
				Hepatic	500			
Jasper et al. 2012 –Roundup® Original (41% glyphosate and 16% POEA								

Table 2-3. Levels of Significant Exposure to Glyphosate Formulations – Oral

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
Rat (Wistar) 16–18 M	30 days, (PPDs 23–53) (GW)	0, 5, 50, 250	BW, DX, HP, OF, OW	Bd Wt	250 M			
				Endocr		5 M		Decreased serum testosterone
				Develop		5 M		Decreased epithelial thickness and increased luminal diameter in seminiferous tubules

Romano et al. 2010 – Roundup Transorb® (648 g/L isopropylamine salt of glyphosate and 594 g/L inerts)

Bd Wt or BW = body weight; CS = clinical signs; Develop = developmental; DX = developmental toxicity; EA = enzyme activity; Endocr = endocrine; F = female(s); FI = food intake; FX = fetal toxicity; Gastro = gastrointestinal; GD = gestation day; GN = gross necropsy; GW = gavage in water vehicle; HE = hematology; Hemato = hematological; HP = histopathology; IT = intratracheal; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); MX = maternal toxicity; NOAEL = no observed-adverse-effect level; NS = not specified; OF = organ function; OW = organ weight; POEA = polyoxyethyleneamine; PPD = post-parturition day; Repro = reproductive; TG = teratogenicity; W = water vehicle; WI = water intake

Table 2-4. Levels of Significant Exposure to Glyphosate Technical – Dermal

Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL	Less serious LOAEL	Serious LOAEL	Effect
INTERMEDIATE EXPOSURE								
Rabbit (New Zealand) 10 M, 10 F	21 days, 5 days/week, 6 hours/day	0, 100, 1,000, 5,000	BC, BW, CS, EA, FI, GN, HE, HP, LE, OW	Bd Wt	5,000			
				Hemato	5,000			
				Hepatic	5,000			
				Dermal	1,000	5,000		Very slight erythema and edema at application site

EPA 1992c – glyphosate technical, purity not specified

BC = biochemistry; BW or Bd wt = body weight; CS = clinical signs; EA = enzyme activity; F = female(s); FI = food intake; GN = gross necropsy; HE = hematology; Hemato = hematological; HP = histopathology; LE = lethality; LOAEL = lowest-observed-adverse-effect level; M = male(s); NOAEL = no observed-adverse-effect level; OW = organ weight

2.2 DEATH

Several case report series have reported deaths in individuals intentionally ingesting glyphosate products (Chen et al. 2009; Kim et al. 2014; Roberts et al. 2010; Sawada et al. 1988; Talbot et al. 1991; Tominack et al. 1991). The predominant cause of death was often shock (hypovolemic or cardiogenic), hypotension, and respiratory failure, often due to aspiration (Chen et al. 2009; Kim et al. 2014; Talbot et al. 1991).

An acute oral LD_{50} value of 4,320 mg/kg/day was reported following single oral dosing of rats with glyphosate technical (EPA 1992b). In a developmental toxicity study, 6/25 pregnant rats died during oral dosing of glyphosate technical at 3,500 mg/kg/day; there were no deaths during treatment at 1,000 mg/kg/day (EPA 1992e). No adequate sources were located regarding death in laboratory animals exposed to glyphosate technical by inhalation or dermal routes.

In a study that employed oral dosing of pregnant rats with Roundup®, 8/15 dams died during the first 8 days of treatment at 1,000 mg/kg/day glyphosate (Dallegrave et al. 2003). No deaths occurred in a 4-week study of rats intermittently exposed to Roundup® at exposure levels as high as 360 mg/m^3 (approximately 36 mg Roundup®/m^3) (EPA 1985c). No adequate sources were located regarding death in laboratory animals exposed to glyphosate formulations by the dermal route.

2.3 BODY WEIGHT

Oral exposure of rats to glyphosate technical at relatively high doses resulted in significant effects on body weight and/or body weight gain. Pregnant rats gavaged at 3,500 mg/kg/day during GDs 6–19 exhibited as much as 28.5% lower mean body weight gain than controls (EPA 1992e). Body weight gain was 12–18% less than that of controls in two generations of parental male and female rats exposed via the diet for 14–19 weeks at 2,219 or 3,134 mg/kg/day, respectively (EPA 1992a). No treatment-related effects on body weight were seen among young female mice treated for 28 days at estimated doses up to 1,447.5 mg/kg/day (EPA 2013b). In 13-week oral studies, body weight and/or body weight gain among rats and mice at oral doses in the range of 2,273–11,977 mg/kg/day were 10–18% less than controls (NTP 1992). In a 2-year study, female rats dosed at 1,183 mg/kg/day exhibited 13% lower mean body weight than controls at treatment week 81 (EPA 1991a). There was no evidence of treatment-related effects on body weight among laboratory animals receiving oral doses of glyphosate technical at ≤1,000 mg/kg/day during acute-, intermediate-, or chronic-duration exposure (EPA 1986a, 1987, 1991a, 1991b, 1992a, 1992d, 1992e, 1992f, 1992g, 2013a, 2013b, 2017b).

No significant treatment-related effects on body weight were observed among rabbits administered repeated dermal applications of glyphosate technical at doses in the range of 100–5,000 mg/kg/application for 21 days (EPA 1992c).

No significant body weight effects occurred in a 4-week study of rats intermittently exposed to Roundup® at exposure levels as high as 360 mg/m^3 (approximately 36 mg Roundup®/m^3) (EPA 1985c). Several studies evaluated effects of oral exposure to glyphosate formulations on body weight. Limited results indicate that mice may be more sensitive than rats to body weight effects from repeated oral exposure to glyphosate formulations. Seriously-depressed mean body weight gain (60–66% less than controls) was reported for albino Swiss mice gavaged with Roundup Original® at 50 mg/kg/day for 15 days and approximately 10% body weight loss for mice dosed at 500 mg/kg/day (Jasper et al. 2012). No significant effects on body weight were observed among Wistar rats gavaged with Roundup® at 56 or 560 mg/kg/day for up to 13 weeks (Caglar and Kolankaya 2008), pregnant Wistar rats gavaged with Roundup® at 1,000 mg/kg/day during GDs 6–15 (Dallegrave et al. 2003), or maternal Wistar rats gavaged with Roundup® at 50–450 mg/kg/day during gestation and lactation (Dallegrave et al. 2007). No effects on body weight were observed among male Wistar rats gavaged with Roundup Transorb® at 250 mg/kg/day during postnatal days (PNDs) 23–53 (Romano et al. 2010).

2.4 RESPIRATORY

As summarized in Table 2-5, several investigations of the Agricultural Health Study participants have examined the possible associations between use of glyphosate-containing products and increased risk of rhinitis, wheezing, atopic asthma, allergic asthma, or chronic bronchitis (Hoppin et al. 2002, 2006a, 2006b, 2007, 2008, 2009; Slager et al. 2009, 2010). No associations were found for diagnosed chronic bronchitis (Hoppin et al. 2007) or for wheezing after adjusting for confounding exposure to other pesticides (Hoppin et al. 2002, 2006a, 2006b). Current rhinitis was associated with glyphosate use among commercial applicators (Slager et al. 2009) and farmers (Slager et al. 2010), but no relationship between risk and the number of days of use per year was found among the commercial applicators (Slager et al. 2009). An association between glyphosate use and the risk of atopic asthma was found among farm women, but there was no association with nonatopic asthma (Hoppin et al. 2008). No associations were found between glyphosate use by male farmers and risk of allergic or nonallergic asthma (Hoppin et al.

Table 2-5. Noncancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study population	Exposure	Outcomes
Respiratory		
Hoppin et al. 2002 Cohort study of 20,468 participants in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use and application frequency categories **Logistic regression adjustments:** age, state, smoking history, asthma-atopy status	Wheeze, self-reported OR 1.05 (0.95–1.17), p=0.04 for trend of increasing exposure days
Hoppin et al. 2006a Prospective cohort study of 20,175 participants in the Agricullural Health Study in Iowa and North Carolina (17,920 farmers and 2,255 commercial pesticide applicators)	**Exposure:** glyphosate ever use in the year prior to enrollment **Logistic regression adjustments:** age, state, smoking history, BMI	Wheeze, self-reported OR 1.05 (0.94–1.17), farmers OR 1.14 (0.83–1.57), applicators
Hoppin et al. 2006b Cohort study of 2,255 commercial pesticide applicators participating in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use in the year prior to enrollment **Logistic regression adjustments:** age, smoking status, asthma and atopy history, BMI	Wheeze, self-reported OR 1.38 (1.03–1.86) OR 1.14 (0.83–1.57), with adjustment for use of chlorimuron-ethyl pesticide
Hoppin et al. 2007 Prospective cohort study of 20,908 participants in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use **Logistic regression adjustments:** age, state, sex, smoking (pack-years)	Chronic bronchitis OR 0.99 (0.82–1.19)
Hoppin et al. 2008 Prospective cohort study of 25,814 farm women participating in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use **Logistic regression adjustments:** age, state, smoking status, "grew up on farm"	Atopic asthma OR 1.31 (1.02–1.67) Nonatopic asthma OR 1.13 (0.92–1.39)
Hoppin et al. 2009 Prospective cohort study of 19,704 male farmers participating in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use **Logistic regression adjustments:** age, state, smoking status, BMI	Allergic asthma OR 1.37 (0.86–2.17) Nonallergic asthma OR 1.15 (0.87–1.51)

Table 2-5. Noncancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study population	Exposure	Outcomes
Slager et al. 2009 Prospective cohort study of 2,245 commercial applicators participating in the Agricultural Health Study in Iowa	**Exposure:** any glyphosate use and application frequency categories during the past year **Logistic regression adjustments:** age, education, "growing up on farm"	Current rhinitis OR 1.32 (1.08–1.61), p=0.735 for trend for increasing use days per year
Slager et al. 2010 Prospective cohort study of 19,565 farmers participating in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** any glyphosate use and application frequency categories during the past year **Logistic regression adjustments:** age; race; education; state; BMI; currently working on farm; years mixing pesticides, repairing engines or pesticide equipment, welding, painting, handling stored grain or hay, working in swine areas, working with hogs or other farm animals, butchering animals, and growing cabbage, Christmas trees, field corn, sweet corn, and hay	Current rhinitis OR 1.09 (1.05–1.13)
Cardiovascular Effects		
Dayton et al. 2010 Case control study of 168 cases of nonfatal myocardial infarction and 22,257 controls in women in Iowa and North Carolina participating in the Agricultural Health Study	**Exposure:** glyphosate ever use **Logistic regression adjustments:** age, BMI, smoking, state	Nonfatal myocardial infarction OR 0.8 (0.6–1.2)
Mills et al. 2009 Prospective study of male participants in the Agricultural Health Study in Iowa and North Carolina (n=54,069 for fatal myocardial infarction and 32,024 for nonfatal incidence)	**Exposure:** glyphosate ever use **Cox proportional regression adjustments:** age, state, smoking, BMI (nonfatal analysis only)	Fatal myocardial infarction HR 0.99 (0.80–1.23) Nonfatal myocardial infarction HR 1.10 (0.93–1.31)

Table 2-5. Noncancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study population	Exposure	Outcomes
Musculoskeletal Effects		
De Roos et al. 2005b Nested case control study of 135 cases of physician-confirmed rheumatoid arthritis and 675 controls participating in the Agricultural Health Study in Iowa and North Carolina (female participants only)	**Exposure:** glyphosate ever use **Unconditional logistic regression adjustments:** birth date, state	Rheumatoid arthritis OR 1.2 (0.8–1.8)
Parks et al. 2016 Nested case-control study of cases of physician-confirmed rheumatoid arthritis or self-reported use of disease modifying antirheumatic drugs and noncases participating in the Agricultural Health Study in Iowa and North Carolina (female spouses of licensed pesticide applicators only); enrolled between 1993 and 1997 and followed through 2010	**Exposure:** glyphosate ever use **Logistic regression adjustments:** age, state, pack-years smoking	Rheumatoid arthritis OR 1.2 (0.95–1.6); based on 100 prevalent cases OR 1.4 (1.0–2.0); based on 54 incident cases
Dermal Effects		
Maibach 1986 Experimental study of 24 males and females	**Exposure:** 0.1 mL applied to intact and Draize-type abraded skin; patch removed after 24 hours	No skin irritation 24 or 48 hours after application to intact skin Irritancy scores 24 hours after application to abraded skin were negative in 10 subjects, equivocal in 4 subjects and erythema was noted in 10 subjects; at 48 hours, the scores were negative in 10 subjects, equivocal in 6 subjects, and erythema was noted in 8 subjects
Maibach 1986 Experimental study of 23 males and females	**Exposure:** 0.1 mL applied 5 days/week for 21 days	The average score was 1.4 where a score of 1 indicates erythema and 2 indicates erythema and induration; none of the subjects reported burning, stinging, or itching from the test compound

Table 2-5. Noncancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study population	Exposure	Outcomes
Maibach 1986 Experimental study of 204 males and females	**Exposure:** 0.2 mL applied to 3 days/week for 3 weeks with patches remaining in place for 48–72 hours; a challenge patch was applied after a 2-week rest period	No skin irritation was observed
Maibach 1986 Experimental study of 15 males and females	**Exposure:** Full-strength glyphosate was applied to skin stripped of the stratum corneum; the test site received irradiation with ultraviolet A and ultraviolet B light	No positive results for photoirritation or photosensitization were found
Ocular Effects		
Kirrane et al. 2005 Prospective study of 31,173 female spouses of commercial pesticide applicators participating in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use **Hierarchical regression adjustments:** age, state	Retinal degeneration OR 1.1 (0.8–1.5)
Endocrine Effects		
Goldner et al. 2010 Prospective study of 16,529 participants (female spouses only) in the Agricultural Health Study in Iowa and North Carolina Thyroid disease was self-reported clinically diagnosed	**Exposure:** glyphosate ever use **Polytomous logistic regression adjustments:** age, education, smoking status, hormone replacement therapy, BMI	Hyperthyroid disease OR 0.98 (0.78–1.2) Hypothyroid disease OR 1.0 (0.91–1.2) Other thyroid disease OR 0.97 (0.81–1.2)
Neurological Effects		
Kamel et al. 2007 Case control study of cases of self-reported Parkinson's disease (n=83 prevalent cases and 78 incident cases) and controls (n=79,557 prevalent controls and 55,931 incident controls) participating in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use **Logistic regression adjustments:** age, state, type of participant	Parkinson's disease OR 1.0 (0.6–1.7), prevalent disease OR 1.1 (0.6–2.0), incident disease Prevalent disease defined as reporting Parkinson's disease at enrollment and incident disease defined as Parkinson's disease reported at the study follow-up

Table 2-5. Noncancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study population	Exposure	Outcomes
Reproductive Effects		
Curtis et al. 1999 Retrospective cohort study of 2,012 planned pregnancies among participants in the Canadian Ontario Farm Family Health Study	**Exposure:** glyphosate use on the farm **Cox proportional hazard adjustments:** age when beginning to try to conceive, recent oral contraceptive use, men's and women's smoking, and use of other pesticides	Fecundability CFR 0.61 (0.30–1.26), pesticide use on the farm and women reported pesticide activities CFR 1.30 (1.07–1.56), pesticide use on the farm, but no pesticide activities reported by women
Developmental Effects		
Arbuckle et al. 2001 Retrospective cohort study of 2,110 female participants in the Canadian Ontario Farm Family Health Study	**Exposure:** glyphosate use during gestation **Logistic regression adjustments:** none	Spontaneous abortion, preconception exposure OR 1.4 (1.0–2.1), all gestational ages OR 1.1 (0.7–1.9), <12 weeks gestation OR 1.7 (1.0–2.9), >12 weeks gestation Spontaneous abortion, postconception exposure OR 1.1 (0.7–1.7), all gestational ages OR 0.8 (0.4–1.6), <12 weeks gestation OR 1.4 (0.8–2.5), >12 weeks gestation
Garcia et al. 1998 Case control study of 261 cases of congenital malformations and 261 matched controls in Spain	**Exposure:** paternal glyphosate use **Conditional logistic regression adjustments:** paternal age and paternal job and maternal history of spontaneous abortion, twins, drug consumption, heavy smoking, education, occupation	Congenital malformations OR 0.94 (0.37–2.34) for the acute risk period (during 3 months preceding conception or during the first trimester of pregnancy or both for the father and during 1 month preceding conception or during the first trimester of pregnancy or both for the mother)
Garry et al. 2002 Cross sectional study of 695 families and 1,532 children in Minnesota	**Exposure:** glyphosate ever use **Regression adjustments:** maternal age, smoking status, alcohol use, season of conception	ADD/ADHD, parent reported OR 3.6 (1.35–9.65)

Table 2-5. Noncancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study population	Exposure	Outcomes
Rull et al. 2006 Case control study of 731 cases of neural tube defects and 940 controls in California	**Exposure:** maternal residential proximity to glyphosate application (within 1,000 m) **Unconditional logistic regression adjustments:** maternal ethnicity, education, periconceptional smoking, vitamin use	Neural tube defects OR 1.5 (1.0–2.4) OR 1.5 (0.8–2.9) with adjustment for other pesticide exposure
Sathyanarayana et al. 2010 Prospective study of 2,246 women whose most recent singleton birth occurred within 5 years of enrollment in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** maternal glyphosate ever use (n=700) **Linear regression adjustments:** maternal BMI and height, parity, preterm status, state, maternal smoking during pregnancy	Multiple regression estimates of change in birth weight (g) in relation to maternal self-reported glyphosate use (coefficient = 4 g; 95% CI -40 to +48 g) indicate no significant association between birth weight and maternal use of glyphosate
Savitz et al. 1997 Retrospective cohort study of 1,898 couples participating in the Canadian Ontario Farm Family Health Study	**Exposure:** any paternal glyphosate use from 3 months prior to conception through the month of conception **Logistic regression adjustments:** maternal age, parity, maternal and paternal education, income, maternal and paternal off farm job, maternal smoking and alcohol use during pregnancy, conception to interview interval	Miscarriage OR 1.5 (0.8–2.7) Preterm delivery OR 2.4 (0.8–7.9) Small for gestational age OR 0.8 (0.2–2.3)
Other Noncancer Effects		
Montgomery et al. 2008 Prospective study of 33,457 participants (white males only) in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** glyphosate ever use **Logistic regression adjustments:** age, state, BMI	Diabetes incidence OR 0.85 (0.74–0.98)

Table 2-5. Noncancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study population	Exposure	Outcomes
Saldana et al. 2007 Prospective study of 11,273 participants in the Agricultural Health Study in Iowa and North Carolina	**Exposure:** any agricultural glyphosate exposure during the first trimester **Unconditional logistic regression adjustments:** BMI at enrollment, mother's age at pregnancy, parity, race, state, commonly used pesticides by women	Gestational diabetes mellitus OR 0.7 (0.2–1.75)

ADD/ADHD = attention deficit disorder/attention deficit hyperactivity disorder; BMI = body mass index; CFR = conditional fecundability ratio; CI = confidence interval; HR = hazard ratio; OR = odds ratio

2009). It is noted that many of these studies did not account for use of other pesticides. Respiratory failure or distress was reported in about 10–25% of the cases of intentional ingestion of glyphosate products (Lee et al. 2000; Moon and Chun 2010; Tominack et al. 1991).

Available data regarding respiratory effects in laboratory animals exposed to glyphosate are limited. Kumar et al. (2014) reported an inflammatory respiratory response (evidenced by increased eosinophil and neutrophil counts, mast cell degranulation, and production of IL-33, TSLP, IL-13, and IL-5) in anesthetized mice exposed intranasally to glyphosate. Adam et al. (1997) designed a study to evaluate the effects of glyphosate technical (200 mg/kg), glyphosate + POEA (200 and 100 mg/kg, respectively), POEA alone (100 mg/kg), and Roundup® in rats evaluated for 24 hours following intratracheal instillation (Adam et al. 1997). Control rats received normal saline. Obvious clinical signs of adverse pulmonary effects and mortalities occurred in each group except the saline controls. The study authors stated that the pulmonary effects were more severe and lasted longer in rats treated with POEA alone or in combination with glyphosate compared to responses in glyphosate only-treated rats. These results suggest POEA was more acutely toxic than glyphosate to the lungs. No respiratory effects occurred in a 4-week study of rats intermittently exposed to Roundup® at exposure levels as high as 360 mg/m^3 (approximately 36 mg Roundup®/m^3) (EPA 1985c).

2.5 CARDIOVASCULAR

Two studies of Agricultural Health Study participants did not find associations between the use of glyphosate-containing products and the risk of myocardial infarctions (Dayton et al. 2010; Mills et al. 2009); see Table 2-5 for details. In case series reports, abnormal electrocardiogram (EKG) readings have been found in patients ingesting large doses of glyphosate-containing products (Kim et al. 2014; Lee et al. 2000, 2008; Moon and Chun 2010; Talbot et al. 1991). The most commonly reported alterations included prolonged QTc interval and sinus tachycardia. In the most severe poisoning cases, hypotension and shock have been reported (Roberts et al. 2010; Sawada et al. 1988; Tominack et al. 1991).

No data were available regarding evaluation of cardiovascular endpoints in laboratory animals exposed to glyphosate technical or glyphosate formulations by any exposure route.

2.6 GASTROINTESTINAL

Gastrointestinal symptoms are commonly reported in case series reports of patients who ingested glyphosate products. In numerous reports, over 40% of the patients reported nausea/vomiting (Lee et al.

2000, 2008; Roberts et al. 2010; Sawada et al. 1988; Tominack et al. 1991). Other effects reported included abdominal pain (Lee et al. 2000, 2008; Moon and Chun 2010; Roberts et al. 2010; Sawada et al. 1988; Talbot et al. 1991), sore throat (Lee et al. 2000; Tominack et al. 1991), and damage to mucosal tissue in the mouth and esophagus (Chang et al. 1999; Sawada et al. 1988; Talbot et al. 1991; Tominack et al. 1991).

Several studies evaluated effects of glyphosate technical oral exposure in laboratory animals. The most common effect was clinical signs of gastrointestinal disturbances. Such clinical signs are commonly observed in studies of laboratory animals receiving bolus gavage doses of test substances, in which cases the clinical signs may be at least partially the result of the method of gavage dosing. Diarrhea was observed among rats gavaged once with glyphosate technical at 2,000 mg/kg (EPA 2013c). Gastrointestinal disturbances (e.g., soft stool, diarrhea, few feces) were reported among pregnant rats gavaged at 3,500 mg/kg/day during GDs 6–19 (EPA 1992e) and pregnant rabbits gavaged at 350 mg/kg/day during GDs 6–27 (EPA 1992f) or 175 mg/kg/day during GDs 8–20 (EPA 2017b). A slight increase in observations of soft stool and/or diarrhea was noted in the rabbits dosed at 175 mg/kg/day during GDs 6–27 as well (EPA 1992f). Soft stools were observed in rats exposed via the diet for 2 generations at concentrations resulting in estimated doses in the range of 2,219–2,633 and 3,035–3,134 mg/kg/day for parental males and females, respectively (EPA 1992a). Mao et al. (2018) reported that glyphosate added to the drinking water of rat dams from GD 6 through lactation and to F1 offspring up to PND 125 at a concentration resulting in a daily dose of 1.75 mg/kg/day (the U.S. acceptable daily intake [ADI]) resulted in modifications to the gut microbiota in early development, particularly among prepubertal rats. In a 2-year study of rats exposed via the diet (EPA 1991a, 1991b), inflammation of gastric squamous mucosa was observed in females at an estimated dose level of 457 mg/kg/day; there were no signs of gastrointestinal effects in males at estimated doses as high as 940 mg/kg/day. In another chronic-duration oral rat study (EPA 1992d), there were no signs of treatment-related gastrointestinal effects at the highest estimated dose level (31.45–34.02 mg/kg/day). No clinical signs or histopathological evidence of treatment-related gastrointestinal effects were seen among male or female mice exposed via the diet for 24 months at estimated doses as high as 4,945 and 6,069 mg/kg/day, respectively (EPA 1985a, 2015a). Increased incidence of exocrine hyperplasia in the pancreas was reported for male rats receiving glyphosate technical from the diet for up to 2 years at an estimated dose of 1,214 mg/kg/day (EPA 2015c). Increased severity of cytoplasmic changes in salivary gland cells (basophilia and hypertrophy of acinar cells in parotid and submandibular salivary glands) was reported for male and female rats receiving glyphosate from the diet for 13 weeks at 410 and 421 mg/kg/day, respectively (NTP 1992) and other rats similarly treated at 300 mg/kg/day for up to 2 years (EPA 2015c).

Similar effects on salivary glands were observed in male and female mice treated for 13 weeks at much higher doses (1,065 and 2,707 mg/kg/day, respectively; not observed at 507 and 753 mg/kg/day, respectively) (NTP 1992). Although salivary gland cytoplasmic changes were noted in rats at doses <300 mg/kg/day as well, the changes were reported to be only of minimal or mild severity; therefore, they are not considered adverse effects. The toxicological significance of the glyphosate treatment-related effects on salivary glands is uncertain.

Limited information was located regarding gastrointestinal effects in laboratory animals following oral exposure to glyphosate formulations. In a study designed to evaluate the effects of glyphosate technical (2,000 mg/kg), glyphosate + POEA (2,000 and 1,000 mg/kg, respectively), POEA alone (1,000 mg/kg), or Roundup® were administered to rats by gavage, followed by 24 hours of posttreatment observation (Adam et al. 1997). Control rats received normal saline. Two rats in the POEA-only treatment group died. Diarrhea was noted in all groups except the control group. The study authors stated that the groups given POEA or mixtures that included POEA experienced more rapid and severe diarrhea than those given glyphosate alone. These results suggest that POEA was more acutely toxic than glyphosate to the gastrointestinal system. Mao et al. (2018) reported that Roundup® added to the drinking water of rat dams from GD 6 through lactation and to F1 offspring up to PND 125 at a concentration designed to deliver a daily dose of 1.75 mg glyphosate/kg/day (the U.S. glyphosate ADI) resulted in modifications to the gut microbiota in early development, particularly among prepubertal rats.

2.7 HEMATOLOGICAL

No information was located regarding hematological effects in humans exposed to glyphosate-containing products; results from available animal studies do not implicate the hematological system as a sensitive target of glyphosate toxicity. Hematological endpoints were evaluated in chronic-duration oral studies of rats (EPA 1991a, 1991b, 1992d), mice (EPA 2015a), and dogs (EPA 1986a, 1987) exposed to glyphosate technical. There were no apparent treatment-related effects in chronic-duration oral studies of rats, mice, or dogs administered glyphosate technical at oral doses as high as 940–1,183 mg/kg/day for rats (EPA 1991a, 1991b, 1992d), 4,945–6,069 mg/kg/day for mice (EPA 2015a), and 500 mg/kg/day for dogs (EPA 1986a, 1987). Rabbits administered repeated dermal applications of glyphosate technical at doses in the range of 100–5,000 mg/kg/application for 21 days exhibited no evidence of treatment-related hematological effects (EPA 1992c). Small changes in hematological parameters were seen in both male and female rats in the 13-week NTP (1992) study. These were considered to be unremarkable and most likely due to mild dehydration.

Available information regarding hematological effects related to glyphosate formulations is limited. No hematological effects occurred in a 4-week study of rats intermittently exposed to Roundup® at exposure levels as high as 360 mg/m^3 (approximately 36 mg Roundup®/m^3) (EPA 1985c). Decreases in red blood cell count, hematocrit, and hemoglobin, and increases in corpuscular volume and neutrophil count were reported in mice gavaged with Monsanto Roundup® Original for 15 days at 500 mg/kg/day (Jasper et al. 2012).

2.8 MUSCULOSKELETAL

De Roos et al. (2005b) did not find an association between glyphosate use and the risk of rheumatoid arthritis among participants of the Agricultural Health Study. In a subsequent study of female spouses of licensed pesticide applicators, Parks et al. (2016) reported a weakly positive association between spousal use of glyphosate and risk of rheumatoid arthritis. See Table 2-5 for additional study details.

No data were available regarding evaluation of musculoskeletal endpoints in laboratory animals exposed to glyphosate technical or glyphosate formulations by any exposure route.

2.9 HEPATIC

No information was located regarding hepatic effects in humans exposed to glyphosate-containing products. The potential for glyphosate technical to cause liver toxicity was evaluated in studies of rats and mice; there is some evidence that oral doses near or above recommended limit dosing for animal studies (2,000 mg/kg/day) may cause adverse liver effects. In a 13-week rat dietary study of glyphosate technical, increases in liver weight and serum ALT were observed in males at 1,678 mg/kg/day; increased liver weight and increased serum AP, ALT, and bile acids were noted in females at 3,393 mg/kg/day. There were no indications of treatment-related liver effects among male and female rats treated via the diet for 2 generations at estimated doses as high as 1,234–1,273 mg/kg/day (EPA 2013a) or other rats treated for 2 years to doses as high as 940–1,183 mg/kg/day (EPA 1991a, 1991b). Male mice exposed via the diet for 13 weeks at doses ≥2,273 mg/kg/day exhibited increased mean relative liver weight (4–9% greater than controls) in the absence of histopathologic liver lesions; there were no effects on liver weight in similarly-treated female mice at doses up to and including 11,977 mg/kg/day (NTP 1992). Male mice exposed via the diet for 2 years at an estimated dose of 4,945 mg/kg/day exhibited increased incidence of histopathologic central lobular hepatocyte necrosis; there was no evidence of treatment-related liver effects in similarly-treated female mice at an estimated dose of 6,069 mg/kg/day (EPA 2015a). Rabbits

administered repeated dermal applications of glyphosate technical at doses in the range of 100–5,000 mg/kg/application for 21 days exhibited no evidence of treatment-related hepatic effects (EPA 1992c).

Available information regarding hepatic endpoints in animals exposed to glyphosate formulations is limited. No hepatic effects occurred in a 4-week study of rats intermittently exposed to Roundup® at exposure levels as high as 360 mg/m^3 (approximately 36 mg Roundup®/m^3) (EPA 1985c). Increased serum ALT and aspartate aminotransferase (AST) activity and histopathologic liver lesions (increased Kupffer cells in hepatic sinusoids and deposition of reticulin fibers) were seen in male rats treated with Glyphosate-Biocarb® by gavage for 75 days (one dose every 2 days) at 487 mg/kg/dosing (Benedetti et al. 2004).

2.10 RENAL

One case-control study of patients with chronic kidney disease found an increased risk of chronic kidney disease among glyphosate applicators (Jayasumana et al. 2015). However, uncertainty regarding an association between exposure to glyphosate-containing products and risk of chronic kidney disease includes the finding that the applicators were also exposed to high levels of calcium, magnesium, barium, strontium, iron, titanium, and vanadium by drinking water from abandoned wells.

Several studies evaluated possible renal toxicity in laboratory animals treated with glyphosate technical. In a 2-generation reproductive toxicity study (EPA 2013a), slightly increased absolute and relative kidney weights (7–11% greater than controls) were reported among F0 parental female rats dosed at 1,273 mg/kg/day; there was no evidence of histopathologic kidney lesions. Therefore, the slightly increased kidney weight was not considered to represent an adverse effect. During 2 years of dietary treatment of rats, urinalysis revealed increased specific gravity of urine and decreased urinary pH among males treated at an estimated dose of 940 mg/kg/day (NOAEL=362 mg/kg/day); there were no signs of treatment-related renal effects in urinalysis results from females treated at an estimated dose as high as 1,183 mg/kg/day (EPA 1991a, 1991b). Papillary necrosis (males and females) and decreased pH of urine (males only) were observed in a study of rats administered glyphosate in the diet for up to 2 years at estimated doses of 1,214 mg/kg/day (males) and 1,498 mg/kg/day (females); respective NOAELs were 361 and 437 mg/kg/day (EPA 2015c). Another 2-year rat study reported decreased pH of urine among males treated at 1,000 mg/kg/day (NOAEL=300 mg/kg/day); no renal effects were observed in females at doses as high as 1,000 mg/kg/day (EPA 2015c). Female mice treated for 2 years at an estimated dose of

6,069 mg/kg/day exhibited significantly increased incidence of renal proximal tubule epithelial basophilia and hypertrophy (NOAEL=968 mg/kg/day); there was no evidence of renal effects in similarly-treated male mice at doses as high as 4,945 mg/kg/day (EPA 2015a).

Information regarding renal effects in animals exposed to glyphosate formulations is limited. No renal effects occurred in a 4-week study of rats intermittently exposed to Roundup® at exposure levels as high as 360 mg/m^3 (approximately 36 mg Roundup®/m^3) (EPA 1985c). Histopathologic kidney lesions (necrotic and apoptotic cells, localized primarily in tubular epithelium of the proximal straight tubule and thick ascending limb of the loop of Henle) were reported in male rats gavaged once with Concentrate Roundup® Weedkiller at dose levels ranging from 250 to 2,500 mg/kg (Wunnapuk et al. 2014). There is some uncertainty regarding the role of glyphosate in the reported effects.

2.11 DERMAL

One study evaluated the potential dermal toxicity of glyphosate in humans. In an experimental study (see Table 2-5), a single application of Roundup® to intact skin for 24 hours did not result in irritation (Maibach 1986). When applied to abraded skin, erythema was noted in 42% of the subjects after 24 hours. Mild skin irritation was observed in a repeated exposure test study (Maibach 1986). No skin irritation was observed in a Draize skin sensitization test or in a photosensitivity/photoirritation test (Maibach 1986).

Available information regarding dermal effects in animals is limited. Minor dermal irritation was reported in response to dermally-applied glyphosate technical. At the application site, very slight erythema and edema were observed in rabbits during 21 days of repeated dermal application of glyphosate technical at 5,000 mg/kg/application; no dermal effects were seen at doses ≤1,000 mg/kg/application (EPA 1992c). According to EPA (1993), glyphosate is considered a slight dermal irritant following acute dermal application.

2.12 OCULAR

In a study of wives of commercial pesticide applicators, no association was found between glyphosate use among the wives and retinal degeneration (Kirrane et al. 2005); see Table 2-5 for details. In a case series report of 1,513 ocular exposures to glyphosate, minor symptoms (primarily transient irritation) were observed in 70% of the cases; most (99%) complained of eye pain (Acquavella et al. 1999). Moderate effects, such as persistent irritation or low-grade corneal burns or abrasions, were observed in about 2% of

the cases. Among the cases with moderate effects, 93% reported eye pain, 20% reported lacrimation, and 27% reported blurred vision.

Two chronic-duration oral studies included ophthalmoscopic examinations of laboratory animals exposed to glyphosate technical. EPA (1991a, 1991b) reported significantly increased incidence of lens abnormalities in male rats treated via the diet for 2 years at an estimated dose of 940 mg/kg/day; there were no indications of a treatment-related ocular effect in female rats at the highest estimated dose level (1,183 mg/kg/day). No signs of treatment-related ocular effects were seen among dogs treated via capsule for 1 year at estimated doses as high as 500 mg/kg/day (EPA 1986a). According to EPA (1993), glyphosate is considered mildly irritating to the eye following ocular instillation. According to FAO and WHO (2016), glyphosate was moderate to severely irritating to the rabbit eye. EFSA (2015) stated that glyphosate acid was a severe ocular irritant, but that salts of glyphosate do not require classification as ocular irritants. There were no signs of exposure-related effects in ophthalmologic examinations of rats intermittently exposed to Roundup® for 4 weeks at exposure levels as high as 360 mg/m^3 (approximately 36 mg Roundup®/m^3) (EPA 1985c).

2.13 ENDOCRINE

Available human information regarding possible associations between exposure to glyphosate-containing products and risk of endocrinological effects is limited to results from one study that reported no associations between any glyphosate exposure and the risks of thyroid diseases (Table 2-5) in the female spouses of Agricultural Health Study participants (Goldner et al. 2010).

In a weight-of-evidence approach to evaluate the potential for glyphosate to affect the endocrine system, EPA (2015b) subjected glyphosate to the Endocrine Disruptor Screening Program Tier 1 (a battery of *in vitro* assays designed assist in evaluation of the potential for a substance to interact with estrogen, androgen, or thyroid signaling pathways). EPA evaluated results from the battery of *in vitro* assays and relevant laboratory mammalian and wildlife studies. Using this approach, EPA determined that there is no convincing evidence of potential interaction between glyphosate and estrogen, androgen, or thyroid pathways in mammals or wildlife. Included in the evaluation of the estrogen pathway were estrogen receptor (ER) binding assays, an ER transactivation assay, aromatase and steroidogenesis assays, a fish short-term reproduction assay, and mammalian and wildlife studies that assessed female reproductive parameters. Included in the evaluation of the androgen pathway were androgen receptor (AR) binding and steroidogenesis assays, a fish short-term reproduction assay, Hershberger and male pubertal assays,

an AR transactivation assay, and mammalian and wildlife studies that assessed male reproductive parameters. Included in the evaluation of the thyroid pathway were male and female pubertal assays, an amphibian metamorphosis assay, and mammalian and wildlife studies that assessed thyroid parameters Refer to EPA (2015b) for study summaries and EPA (2015d) for DERs from most studies that contributed to EPA's conclusions regarding the potential for glyphosate to affect the endocrine system.

Limited information was located regarding the potential for glyphosate formulations to affect the endocrine system. Romano et al. (2010) reported dose-related 30–50% decreased serum testosterone in young male rats gavaged with Roundup Transorb® at 5–250 mg/kg/day during postpartum days 23–53. Romano et al. (2012) implicated disruption of gonadotropin expression as a mechanism of action for glyphosate-induced effects on male rat sexual development.

2.14 IMMUNOLOGICAL

Studies examining possible associations between glyphosate exposure and asthma risk or rheumatoid arthritis risk are discussed in Sections 2.4 and 2.8, respectively.

Limited information is available regarding immunological effects. There was no evidence of treatment-related effects on spleen or thymus of mice administered glyphosate technical in the diet for 28 days at estimated doses as high as 1,447.5 mg/kg/day and no evidence of treatment-related effects on splenic anti-sheep red blood cell (SRBC) anti-body forming cell (AFC) responses to SRBC (EPA 2013b). EPA (1992d) reported significantly increased incidences of lymphocytic hyperplasia in the thymus from female rats administered glyphosate technical in the diet for up to 26 months at doses of 3.37, 11.22, and 34.02 mg/kg/day (13/32, 18/37, and 17/34, respectively, versus 5/25 controls). However, EPA (1992d) did not consider the lesion to be compound-related because the lesion occurs spontaneously in older rats and is quite variable in the thymus, there was no apparent effect on lymphocytes in the spleen (a much less variable indicator for lymphocytic hyperplasia), and the severity of the lesion was similar among controls and glyphosate-treated groups. Kumar et al. (2014) reported an inflammatory respiratory response (evidenced by increased eosinophil and neutrophil counts, mast cell degranulation, and production of IL-33, TSLP, IL-13, and IL-5) in anesthetized mice exposed intranasally to glyphosate.

2.15 NEUROLOGICAL

Available information regarding possible associations between exposure to glyphosate-containing products and risk of neurological effects in humans is limited to a single case-control study that did not

find an association between glyphosate exposure and Parkinson's disease (see Table 2-5 for details) (Kamel et al. 2007).

In one animal study, rats were administered glyphosate technical once by gavage at up to 2,000 mg/kg and observed for up to 2 weeks postdosing. In a separate study, rats were treated via the diet for 13 weeks at doses as high as 1,547–1,631 mg/kg/day (EPA 2013c). There was no evidence of treatment-related neurotoxicity in either study as assessed by clinical signs, functional observational battery, motor activity testing, and gross and histopathologic examination of brain and peripheral nervous tissue. However, clinical signs included decreased activity, subdued behavior, and hunched posture.

2.16 REPRODUCTIVE

No association between glyphosate use and fecundability was found among women living at farms in which pesticides were used and were involved in pesticide activities (Curtis et al. 1999). This study also reported an association with improved fecundability when the women were not involved in pesticide activities; see Table 2-5 for additional information.

Increased incidence of prostatitis was reported among male rats receiving glyphosate technical from the diet for up to 2 years at estimated doses of ≥361 or 1,214 mg/kg/day (EPA 2015c). There was no evidence of treatment-related reproductive effects among parental male or female rats administered glyphosate technical in the diet for 2 generations at estimated doses as high as 1,234–3,134 mg/kg/day (EPA 1992a, 2013a). Cassault-Meyer et al. (2014) reported increased abnormal sperm morphology in rats receiving Roundup® Grand Travaux Plus from the drinking water for 8 days at 640 mg/kg/day (the only dose level tested). See Section 2.17 for information regarding treatment-related effects on the reproductive system of male rats exposed to glyphosate formulations during *in utero* and/or postnatal development.

2.17 DEVELOPMENTAL

Several epidemiology studies have examined possible associations between glyphosate use and developmental toxicity; these studies are summarized in Table 2-5. Given that only one study examined each endpoint and the lack of quantification of glyphosate exposure across studies, these results were not considered sufficient for drawing conclusions on the risk of developmental toxicity associated with glyphosate exposure in humans. Arbuckle et al. (2001) reported a positive association between maternal preconception exposure to glyphosate and increased risk of spontaneous abortion (miscarriage). Garry et

al. (2002) reported a positive association between glyphosate exposure and parent-reported attention deficit disorder/attention deficit hyperactivity disorder (ADD/ADHD). No associations were found between paternal exposure and risk of miscarriages (Savitz et al. 1997), preterm delivery (Savitz et al. 1997), small for gestational age risk (Savitz et al. 1997), or congenital malformations (Garcia et al. 1998). Similarly, no associations were found between maternal glyphosate exposure and birth weight (Sathyanarayana et al. 2010) or neural tube defects (Rull et al. 2006).

Developmental endpoints were evaluated in animals orally exposed to glyphosate technical. Depressed weight and increased incidence of unossified sternebrae were observed in fetuses from rat dams treated by gavage at 3,500 mg/kg/day during GDs 6–19 (EPA 1992e). Increased incidence of kidney tubular dilation was reported for F3b male weanlings in a 3-generation study of glyphosate technical (98.7% purity) administered to male and female Sprague-Dawley rats in the diet at an estimated dose level of 30 mg/kg/day; the reported NOAEL was 10 mg/kg/day (EPA 1992g). However, there were no signs of treatment-related effects on kidneys of rat offspring in two subsequent 2-generation rat studies at dose levels up to 1,234 mg/kg/day (EPA 2013a) or 3,134 mg/kg/day (EPA 1992a). Therefore, the finding of increased incidence of kidney tubular dilation in the 3-generation rat study (EPA 1992g) was considered a spurious result rather than a glyphosate-induced adverse developmental effect. In one 2-generation oral rat study, exposure via the diet at an estimated dose level of 1,234 mg/kg/day resulted in delayed preputial separation in male pups (EPA 2013a). In the other 2-generation study, the highest dose level (3,134 mg/kg/day) resulted in up to 14–20% depressed pup body weight and/or body weight gain during the lactation period (EPA 1992a). There were no apparent treatment-related developmental effects in a study of rabbits treated by gavage at up to 350 mg/kg/day during GDs 6–27 (EPA 1992f). Depressed mean fetal weight (8% less than controls) was noted in a study of pregnant rabbits administered glyphosate acid at 300 mg/kg/day during GDs 8–20 (EPA 2017b). However, on a per litter basis, there was no statistically significant difference between controls and glyphosate-treated groups. Therefore, the 300 mg/kg/day dose level is considered a NOAEL for fetal body weight.

Developmental endpoints were evaluated in three open-literature studies that employed oral exposure to glyphosate formulations. The specific role of glyphosate in the reported results is uncertain. Dallegrave et al. (2003) observed an increased incidence of skeletal malformations in fetuses from rat dams gavaged with Roundup® at 500 mg/kg/day during GDs 6–15. Dallegrave et al. (2007) reported decreased sperm production and histopathologic testicular lesions in offspring of rat dams gavaged with Roundup® at 50 mg/kg/day during gestation and lactation. Romano et al. (2010) reported decreased epithelial thickness and increased luminal diameter in seminiferous tubules of male rat pups treated with Roundup

Transorb® by gavage at 5 mg/kg/day on postpartum days 23–53 and delayed preputial separation at a dose level of 50 mg/kg/day.

2.18 OTHER NONCANCER

No associations were found between glyphosate exposure and increased risks of diabetes (Montgomery et al. 2008) or gestational diabetes (Saldana et al. 2007) in epidemiology studies (see Table 2-5). Metabolic acidosis (Kim et al. 2014; Lee et al. 2008; Moon and Chun 2010; Tominack et al. 1991), hyperkalemia (Kim et al. 2014; Lee et al. 2008; Moon and Chun 2010), and acute pancreatitis (Hsiao et al. 2008; Kim et al. 2014; Moon and Chun 2010) have been reported in case series of individuals ingesting glyphosate; metabolic acidosis was typically reported in >35% of the cases.

Hypothermia was reported among rats following single gavage dosing of glyphosate technical at 2,000 mg/kg (EPA 2013c).

2.19 CANCER

Meta-Analyses of Epidemiological Studies

Lymphohematopoietic Cancers. From 2014 to 2016, several meta-analyses were conducted for lymphohematopoietic cancers. The results of these analyses are presented in Table 2-6. The primary literature used in these meta-analyses is discussed later in this section.

Schinasi and Leon (2014) conducted a systematic review and meta-analysis of 21 pesticide active ingredients and chemical groups including glyphosate. The authors reported a positive association between glyphosate use and B-cell lymphoma based on two studies (meta-relative risk [RR] 2.0; 95% confidence interval [CI] 1.1–3.6) and a positive association between glyphosate use and non-Hodgkin's lymphoma (NHL) based on six studies (meta RR 1.5; 95% CI 1.1–2.0).

Chang and Delzell (2016) performed meta-analyses for NHL subtypes (diffuse large B-cell lymphoma, B-cell lymphoma, chronic lymphocytic leukemia/small lymphocytic leukemia [CLL/SLL], and hairy-cell leukemia), as well as other types of lymphohematopoietic cancers (leukemia, multiple myeloma, and Hodgkin's lymphoma). The authors reported a positive association between glyphosate use and the risk of NHL (meta RR 1.3; 95% CI 1.0–1.6; six studies), multiple myeloma (meta RR 1.4; 95% CI 1.0–1.9; four studies), and the NHL subtype B-cell lymphoma (meta RR 2.0; 95% CI 1.1–3.6; two studies). The

Table 2-6. Summary of Meta-Analyses of Results from Studies Examining Possible Association Between Self-Reported Use of Glyphosate and Lymphohematopoietic Cancers

Outcome	Studies included in analysis	Number of participants	Number reporting glyphosate use	Meta-analysis[a] relative risk (95% CI)	Reference
Non-Hodgkin's lymphoma	De Roos et al. 2003 De Roos 2005a Eriksson et al. 2008 Hardell et al. 2002 McDuffie et al. 2001 Orsi et al. 2009	650 cases/1,933 controls 54,315 1,163 cases/1,016 controls 515 cases/1,141 controls 517 cases/1,506 controls 244 cases/436 controls	36 cases/61 controls 71 cases 29 cases/18 controls 8 cases/8 controls 51 cases/133 controls 12 cases/24 controls	1.5 (1.1–2.0) I^2=32.7%	Schinasi and Leon 2014
Non-Hodgkin's lymphoma	De Roos et al. 2003 De Roos 2005a Eriksson et al. 2008 Hardell et al. 2002 McDuffie et al. 2001 Orsi et al. 2009	Not stated 54,315 910 cases/1,016 controls 404 cases/741 controls 517 cases/1,506 controls 244 cases/456 controls	Not stated Not stated 29 cases 8 cases 51 cases 12 cases	1.3 (1.03–1.65) I^2=0.0%, p=0.589 for heterogeneity	IARC 2017
Non-Hodgkin's lymphoma	De Roos et al. 2003 De Roos 2005a Eriksson et al. 2008 Hardell et al. 2002 McDuffie et al. 2001 Orsi et al. 2009	650 cases/1,933 controls 49,211 995 cases/1,016 controls 515 cases/1,141 controls 517 cases/1,506 controls 244 cases/456 controls	36 cases/61 controls 71 cases 29 cases/18 controls 8 cases/8 controls 51 cases/133 controls 12 cases/24 controls	1.3 (1.0–1.6) I^2=0.0%, p=0.84 for heterogeneity	Chang and Delzell 2016
B-cell lymphoma	Cocco et al. 2013 Eriksson et al. 2008	2,348 cases/2,462 controls 1,163 cases/1,016 controls	4 cases/2 controls Not stated	2.0 (1.1–3.6) I^2=0.0%, p=0.58 for heterogeneity	Chang and Delzell 2016; Schinasi and Leon 2014
Leukemia	Brown et al. 1990 De Roos et al. 2005a Kaufman et al. 2009	578 cases/1,245 controls 49,211 180 cases/756 controls	15 cases/49 controls 43 cases 1 case/3 controls	1.0 (0.6–1.5) I^2=0.0%[a], p=0.92 for heterogeneity	Chang and Delzell 2016
Multiple myeloma	Brown et al. 1993 De Roos et al. 2005a Kachuri et al. 2013 Orsi et al. 2009 Pahwa et al. 2012 Sorahan 2015	173 cases/650 controls 19 cases 342 cases/1,357 controls 56 cases/456 controls 32 cases/133 controls 40,719	11 cases/40 controls Not stated 32 cases/131 controls 5 cases/24 controls Not stated 24 cases	1.4 (1.0–1.9) I^2=0.0%, p=0.63 for heterogeneity	Chang and Delzell 2016

Table 2-6. Summary of Meta-Analyses of Results from Studies Examining Possible Association Between Self-Reported Use of Glyphosate and Lymphohematopoietic Cancers

Outcome	Studies included in analysis	Number of participants	Number reporting glyphosate use	Meta-analysis[a] relative risk (95% CI)	Reference
Hodgkin's lymphoma	Karunanayake et al. 2012 Orsi et al. 2009	316 cases/1,506 controls 87 cases/496 controls	38 cases/133 controls 6 cases/24 controls	1.1 (0.7–1.6) I^2=0.0%, p=0.36 for heterogeneity	Chang and Delzell 2016
Diffuse large B-cell lymphoma	Eriksson et al. 2008 Orsi et al. 2009	955 cases/1,016 controls 456 controls	Not stated 5 cases/24 controls	1.1 (0.5–2.3) I^2=0.0%, p=0.79 for heterogeneity	Chang and Delzell 2016
CLL/SLL	Eriksson et al. 2008 Orsi et al. 2009	955 cases/1,016 controls 456 controls	Not stated 2 cases/18 controls	1.3 (0.2–10) I^2=83.7%, p=0.01 for heterogeneity	Chang and Delzell 2016
Follicular lymphoma	Eriksson et al. 2008 Orsi et al. 2009	955 cases/1,016 controls 456 controls	Not stated 3 cases/24 controls	1.7 (0.7–3.9) I^2=0.0%, p=0.73 for heterogeneity	Chang and Delzell 2016
Hairy cell leukemia	Orsi et al. 2009 Nordstrom et al. 1998	456 controls 111 cases/400 controls	2 cases/18 controls 4 cases/5 controls	2.5 (0.9–7.3) I^2=0.0%, p=0.63 for heterogeneity	Chang and Delzell 2016

[a]I^2 is a measure of total variance explained by study heterogeneity and measure of inconsistency in results; higher values indicate greater inconsistency.

CI = confidence interval; CLL/SLL = chronic lymphocytic leukemia/small lymphocytic lymphoma

authors concluded that associations were statistically null for Hodgkin's lymphoma (meta RR 1.1; 95% CI 0.7–1.6; two studies), leukemia (meta RR 1.0; 95% CI 0.6–1.5; three studies); and the NHL subtypes diffuse large B-cell lymphoma (meta RR 1.1; 95% CI 0.5–2.3; two studies), CLL/SLL (meta RR 1.3; 95% CI 0.2–10; two studies), follicular lymphoma (meta RR 1.7; 95% CI 0.7–3.9; two studies), and hairy cell leukemia (meta RR 2.5; 95% CI 0.9–7.3; two studies). Some of the RR CIs were wide, indicating uncertainty in the point estimate.

The IARC Working Group conducted a meta-analysis for NHL using the same six studies as Schinasi and Leon (2014) and Chang and Delzell (2016). The Working Group reanalyzed the data, but used the most fully adjusted risk estimates for the studies by Hardell et al. (2002) and Eriksson et al. (2008) and estimated a slightly lower meta-analysis relative risk (meta RR 1.3; 95% CI 1.03–1.65) (IARC 2017).

Epidemiological Studies

A number of case-control and prospective cohort epidemiology studies have examined possible associations between use of glyphosate-containing compounds and increased cancer risks. Detailed overviews—including a description of the exposure metric used, the results, and the conclusions and limitations as reported by the study authors—are presented in Table 2-7 for solid tumor types and Table 2-8 for lymphohematopoietic cancers.

The majority of the studies examined individuals who were occupationally exposed to pesticides and used self-reported or proxy-reported (ever/never use of glyphosate-containing compounds) use as the marker of exposure. A few studies examined potential cancer risk among family members (i.e., wife and children) of pesticide applicators. The cohort studies utilized data on participants from the Agricultural Health Study, a prospective study of cancer and other health outcomes. The cohort consisted of >89,000 licensed pesticide applicators and their spouses (52,394 applicators and 32,345 spouses) who were recruited between 1993 and 1997 from Iowa and North Carolina. Study limitations included self-reported exposure information, few cases for many of the cancer subtypes, limited information regarding the timing and duration of exposure, and recall bias.

Solid Tumors. The epidemiological studies on the association between glyphosate use and solid-type tumors are presented in Table 2-7. Overall, these studies did not detect a statistically significant association between glyphosate use and all cancer types studied, including melanoma, childhood cancers,

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Andreotti et al. 2018 Prospective cohort study of 54,251 licensed pesticide applicators (97% white, 97% male) recruited between 1993 and 1997 in Iowa and North Carolina from the Agricultural Health Study to evaluate agricultural exposure to 50 pesticides (including glyphosate) and cancer incidence cases. 44,932 participants reported ever use of glyphosate, including 5,779 participants with incident cancer cases.	Exposure: Self-reported ever/never use of any glyphosate pesticides, lifetime days of glyphosate use (days per year x number of years), and intensity-weighted lifetime days (lifetime days x intensity score) at enrollment (1993–1997) or follow-up (1999–2005). Intensity-weighted lifetime days of glyphosate use was categorized into quartiles, tertiles, or the median, such that there were at least five exposed cases in each category. Outcome: Incident cancer diagnoses ascertained via linkage to cancer registries in Iowa (enrollment through 2013) and North Carolina (enrollment through 2012). Data analysis: Poisson regression Adjustments: Age, cigarette smoking status, alcohol drinks per month, family history of any cancer, state of recruitment, and the five pesticides (atrazine, alachlor, metolachlor, trifluralin, and 2,4-D). Confounders considered included BMI and pack-years of cigarettes smoked.	Oral cavity: Q4: RR 0.84 (0.48–1.46) p-trend: 0.54 Colon: Q4: RR 1.01 (0.74–1.38) p-trend: 1.00 Rectum: Q4: RR 0.84 (0.52–1.34) p-trend: 0.43 Pancreas: Q4: RR 1.06 (0.57–1.97) p-trend: 0.14 Lung: Q4: RR 1.00 (0.76–1.33) p-trend: 0.78 Melanoma: Q4: RR 1.17 (0.78–1.74) p-trend: 0.53 Prostate: Q4: RR 0.99 (0.86–1.13) p-trend: 0.89 Testicular: T3: RR 0.57 (0.20–1.67) p-trend: 0.07 Bladder: Q4: RR 1.26 (0.87–1.82) p-trend: 0.42	Conclusions: The authors observed no associations between glyphosate use and overall cancer risk or risk of cancer of the oral cavity, colon, rectum, pancreas, lung, skin, prostate, testes, bladder or kidney. Risk estimates were similar in magnitude between the unlagged and lagged (5 or 20 years) exposure analyses for all sites evaluated. Limitations: Some misclassification of exposure undoubtedly occurred; because many cancer sites were evaluated, there is the possibility that results were observed by chance, and should be interpreted with caution.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
		Kidney: Q4: RR 1.03 (0.66–1.61) p-trend: 0.95	
De Roos et al. 2005a Prospective cohort study of 54,315 certified pesticide applicators (97% male, 97% Caucasian) in Iowa and North Carolina (Agricultural Health Study) to evaluate agricultural exposure to glyphosate and cancer incidence. Among 54,315 subjects included in age-adjusted analyses, 41,035 subjects reported exposure to glyphosate and 13,280 reported no exposure. Number cases (exposed percent) for different cancer sites: All cancers: 2,088 (73.0%) Lung: 204 (72.1%) Oral cavity: 59 (76.3%) Colon: 174 (75.3%) Rectum: 76 (77.6%) Pancreas: 38 (76.3%) Kidney: 63 (73.0%) Bladder: 79 (76.0%) Prostate: 825 (72.5%) Melanoma: 75 (84.0%)	Exposure: Self-reported never/ever use of glyphosate. Cumulative exposure days (CEDs): 1–20 (reference), 21–56, and 57–2,678 days. Intensity weighted exposure days (IWEDs) of 0.1–79.5 (reference), 79.6–337.1, and 337.2–18,241 units. Outcomes/endpoints: Cancer registry files in Iowa and North Carolina for case identification. Incident cases were identified from enrollment to 2001 (median follow-up time: 6.7 years). Data analysis: Poisson regression analyses for all cancers combined and 12 specific cancer sites (with at least 30 cases). Adjustments: Age at enrollment, education, pack-years of cigarette smoking, alcohol consumption, family history of cancer, state of residency, and co-exposure to 10 other pesticides (2,4-D, alachlor, atrazine, metolachlor, trifluralin, benomyl, maneb, paraquat, carbaryl, and diazinon).	All cancers: Ever used: RR 1.0 (0.9–1.2) CED T3: RR 1.0 (0.9–1.1) p-trend: 0.57 IWED T3: RR 0.9 (0.8–1.1) p-trend: 0.35 Lung: Ever used: RR 0.9 (0.6–1.3) CED T3: RR 0.7 (0.4–1.2) p-trend: 0.21 IWED T3: RR 0.6 (0.3–1.0) p-trend: 0.02 Oral cavity: Ever used: RR 1.0 (0.5–1.8) CED T3: RR 0.8 (0.4–1.7) p-trend: 0.66 IWED T3: RR 1.0 (0.5–2.3) p-trend: 0.95 Colon: Ever used: RR 1.4 (0.8–2.2) CED T3: RR 0.9 (0.4–1.7) p-trend: 0.54 IWED T3: RR 1.4 (0.8–2.5) p-trend: 0.10 Rectum: Ever used: RR 1.3 (0.7–2.3)	Conclusions: No association between glyphosate exposure and all cancer incidence or most of the specific cancer subtypes, including NHL. A small number of cases suggested a positive association between multiple myeloma and glyphosate exposure. Limitations: Self-reported exposure information, few cases for many of the cancer subtypes, most applicators were male, there is no information on timing of pesticide use in relation to disease.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
		CED T3: RR 1.1 (0.6–2.3) p-trend: 0.70 IWED T3: RR 0.9 (0.5–1.9) p-trend: 0.82 Pancreas: Ever used: RR 0.7 (0.3–2.0) CED T3: RR 1.3 (0.5–3.6) p-trend: 0.83 IWED T3: RR 0.5 (0.1–1.9) p-trend: 0.06 Kidney: Ever used: RR 1.6 (0.7–3.8) CED T3: RR 0.7 (0.3–1.6) p-trend: 0.34 IWED T3: RR 0.5 (0.2–1.0) p-trend: 0.15 Bladder: Ever used: RR 1.5 (0.7–3.2) CED T3: RR 1.2 (0.6–2.2) p-trend: 0.53 IWED T3: RR 0.8 (0.3–1.8) p-trend: 0.88 Prostate: Ever used: RR 1.1 (0.9–1.3) CED T3: RR 1.1 (0.9–1.3) p-trend: 0.69 IWED T3: RR 1.1 (0.9–1.3) p-trend: 0.60 Melanoma: Ever used: RR 1.6 (0.8–3.0)	

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
		CED T3: RR 0.9 (0.5–1.8) p-trend: 0.77 IWED T3: RR 0.7 (0.3–1.2) p-trend: 0.44	
Engel et al. 2005 Prospective cohort study of 30,454 wives (98% Caucasian) of private pesticide applicators (largely farmers) in Iowa and North Carolina (Agricultural Health Study) to evaluate breast cancer risk in relation to use of individual pesticides by the women themselves or by their husbands. Glyphosate analysis for wife's pesticide use among all wives in the cohort included 82 exposed and 227 unexposed cases (n= 309) and 10,016 exposed and 20,129 (n= 30,145) unexposed controls. Further analysis of husband's pesticide use among wives who reported never having used pesticides themselves included 109 "exposed" (husband used pesticide) and 43 "unexposed" cases and 9,304 "exposed" and 3,993 "unexposed" controls.	Exposure: Self-reported ever/never use of any glyphosate products at enrollment (1993–1997). Husband's information was used as a measure of possible indirect pesticide exposure for their wives. Outcomes/endpoints: Breast cancer incident cases identified through state cancer registries from enrollment to 2000 (mean follow-up period: 4.8 years). Data analysis: Poisson regression Adjustments: Age, race, and state of residence. Confounders considered included BMI, age at menarche, parity, age at first birth, menopausal status, age at menopause, family history of breast cancer, physical activity, smoking, alcohol consumption, fruit and vegetable consumption, and education.	Breast cancer: Wife's pesticide use among all wives in cohort: RR 0.9 (0.7–1.1) Husband's pesticide use among wives who never used pesticides: RR 1.3 (0.8–1.9)	Conclusions: No specific conclusion was given on glyphosate exposure and breast cancer. Limitations: Some associations may have occurred by chance, data on pesticide-specific exposure-response relations were only available for the husband, lack of information on how long each woman had been married to her current partner, limited power to assess associations for less commonly used pesticides, pesticide use was based on self-reporting.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Flower et al. 2004 Prospective and retrospective cohort study of 17,280 children (52% male, 96% Caucasian) of pesticide applicators in Iowa (Agricultural Health Study) to evaluate parental exposure to 50 pesticides (including glyphosate) and childhood cancer risk. Glyphosate analysis included 6,075 children (13 cases) with maternal use and 3,231 children (6 cases) with paternal use of glyphosate.	Exposure: Self-reported parental ever/never use of any glyphosate product by both applicators and spouses at enrollment (1993–1997). Outcomes/endpoints: Childhood cancer cases were both retrospectively and prospectively identified after parental enrollment through Iowa Cancer registries from 1975 to 1998. Data analysis: Multiple logistic regression. Adjustments: Child's age at parent's enrollment. Confounders considered included parental age at child's birth, child's sex, child's birth weight, history of parental smoking, paternal history of cancer, and maternal history of miscarriage.	Childhood cancers: Maternal use (ever): OR 0.61 (0.32–1.16) Paternal use (prenatal): OR 0.84 (0.35–2.34)	Conclusions: No significant associations were observed between maternal (or paternal) pesticide (including glyphosate) application, including increased frequency of application, and risk of childhood cancer risk. Limitations: Small number of cases limits statistical power, maternal use is limited by lack of data on timing of exposure in relation to child's birth, paternal prenatal use constitutes a broad window of exposure and not necessarily just prenatal.

134

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Koutros et al. 2013a, 2013b Prospective cohort study of 54,412 certified pesticide applicators in Iowa and North Carolina (Agricultural Health Study) to evaluate agricultural exposure to 50 pesticides (including glyphosate) and prostate cancer risk. There were 1,962 incident prostate cancer cases, 919 of whom had aggressive prostate cancer. Glyphosate analysis included 1,464 exposed and 498 unexposed cases (n=1,962) and 42,420 exposed and 10,015 unexposed controls (n=52,435).	Exposure: Self-reported ever/never glyphosate use, lifetime days of glyphosate use (years of use x days/year used), intensity-weighted lifetime days of glyphosate use (lifetime days x exposure intensity) at enrollment (1993–1997). Exposure was categorized into non-exposed and quartiles exposure on the basis of the distribution of exposed cases. Outcomes/endpoints: Prostate cancer incidences determined through state cancer registries from enrollment to 2007. Data analysis: Poisson regression. Adjustments: Age at enrollment, race, state, family history of prostate cancer, smoking, fruit servings, and leisure-time physical activity in the winter. Separate glyphosate analyses were conducted by disease aggressiveness and family history of prostate cancer (yes, no).	Cumulative lifetime exposure based on intensity-weighted days: Total prostate cancer: Q4: RR 0.99 (0.86–1.15) Aggressive prostate cancer: Q4: RR 0.94 (0.75–1.18) Total prostate cancer, no family history: Q4: RR 1.02 (0.86–1.21) p-trend: 0.27 Total prostate cancer, with family history: Q4: RR 0.95 (0.64–1.40) p-trend: 0.71	Conclusions: No significant association was found between any specific pesticide (including glyphosate) and risk of total prostate cancer. Limitations: Information on Gleason score of severity was missing for some and not standardized, which most likely led to an underestimation of advanced cases; use of take-home questionnaire could introduce selection bias and exposure misclassification; large number of pesticides investigated so cannot rule out the possibility that some findings may be due to chance.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Koutros et al. 2016 Prospective cohort study of 54,344 male pesticide applicators in Iowa and North Carolina (Agricultural Health Study) to evaluate agricultural exposure to 65 pesticides (including glyphosate) and bladder cancer risk (n=321 incident cases identified). Glyphosate analysis included 248 exposed and 73 unexposed cases (n=321) and 54,023 controls.	Exposure: Self-reported ever/never glyphosate use, lifetime days of glyphosate use (years of use x days/year used), intensity-weighted lifetime days of glyphosate use (lifetime days x exposure intensity) at enrollment (1993–1997). Outcomes/endpoints: Bladder cancer incidences determined through state-based cancer registries from enrollment through 2010 in North Carolina and 2011 in Iowa. Data analysis: Poisson regression. Adjustments: Age, race, state, cigarette smoking, and pipe smoking.	Bladder cancer: Ever use: RR 1.17 (0.78–1.77) Cumulative lifetime exposure based on intensity-weighted days: Overall Q4: RR 1.07 (0.73–1.56) p-trend: 0.99 Stratification by smoking status Never smoker: Q4: RR 1.93 (0.95–3.91) p-trend: 0.03 Former smoker: Q4: RR 1.00 (0.58–1.72) p-trend: 0.67 Current smoker: Q4: RR 0.58 (0.25–1.34) p-trend: 0.17	Conclusions: No specific conclusion given on glyphosate exposure and bladder cancer. Never smokers who were heavy users of the glyphosate had increased risk of bladder cancer. Limitations: Potential for exposure misclassification, findings may be due to chance, due to small number of cases.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Lee et al. 2007 Prospective cohort study of 56,813 certified pesticide applicators (97% male, 97% Caucasian) in Iowa and North Carolina (Agricultural Health Study) to evaluate agricultural exposure to 50 pesticides (including glyphosate) and colorectal cancer risk. Glyphosate analysis included 225 exposed and 67 unexposed for colorectal cancer cases (n=305), 151 exposed and 49 unexposed for colon cancer cases (n=212), and 74 exposed and 18 unexposed for rectal cancers (n=93).	Exposure: Self-reported ever use of any glyphosate pesticides at enrollment (1993–1997). Outcomes/endpoints: Colorectal cancer incidences determined through cancer registries from enrollment to 2002 (mean follow-up period: 7.3 years). Data analysis: Unconditional multivariate logistic regressions. Adjustments: Age, state of residence, smoking history, total pesticide application days to any pesticide. Confounders considered included BMI, race, license type, education level, aspirin intake, family history of colorectal cancer, physical activity, smoking, and intakes of meat, fruits, vegetables, and alcohol.	Colorectal cancer: OR 1.2 (0.9–1.6) Colon cancer: OR 1.0 (0.7–1.5) Rectal cancer: OR 1.6 (0.9–2.9)	Conclusions: No specific conclusion was given on glyphosate exposure and colorectal cancers. Limitations: Since the study examined risks for 50 pesticides, it is possible that some significant findings might occur by chance alone due to the multiple comparisons. Potential recall bias and thus exposure misclassification associated with subjects recalling pesticide use from many years ago.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Andreotti et al. 2009 Nested case-control study of 93 cases of pancreatic cancer (64 applicators and 29 spouses) and 82,503 controls (52,721 applicators and 29,782 spouses) from the Agricultural Health Study, conducted in Iowa and North Carolina, to evaluate the association of pancreatic cancer and use of 24 pesticides (including glyphosate). Glyphosate analysis included 55 exposed and 35 unexposed cases (n= 90) and 48,461 exposed and 31,282 unexposed controls (n= 79,743).	Exposure: Self-reported ever/never use of any glyphosate product for applicators and spouses and intensity-weighted lifetime exposure days for applicators at enrollment (1993–1997). Outcomes/endpoints: Pancreatic cancer incidences identified through state cancer registries from enrollment to 2004 (over 9 years of follow-up time). Data analysis: Unconditional logistic regression. Adjustments: Age, cigarette smoking, diabetes, and subject type for ever/never pesticide exposure (applicator versus spouse).	Pancreatic cancer: Ever/never among applicators and spouses: OR 1.1 (0.6–1.7) Intensity weighted pesticide exposure among applicators: Never: 1.0 (reference) ≤184: 1.9 (0.9–3.8) ≥185: 1.2 (0.6–2.6) p-trend: 0.85	Conclusions: No specific conclusion given on glyphosate exposure and pancreatic cancer Limitations: There was a limited number of exposed cases and limited in generalizability due to predominantly white male study population.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Band et al. 2011 Case-control study on male cancer patients (96.8% Caucasian) in British Columbia, Canada, to evaluate exposure to 139 specific active compounds in pesticides (including glyphosate) and prostate cancer risk. Glyphosate analysis included 25 exposed and 1,128 unexposed cases (n=1,153) and 60 exposed and 3,939 age-matched internal controls (patients with cancer of other primary site) controls (n=3,999).	Exposure: Self-reported ever/never use of glyphosate pesticides from questionnaire. Agricultural job exposure matrix (JEM) was developed for farm workers in British Columbia for the period of 1950–1998. Outcomes/endpoints: Prostate cancer cases identified through British Columbia Cancer Registry for 1983–1990 and histologically confirmed. Data analysis: Conditional logistic regression matched sets of cases and controls. Adjustments: Alcohol consumption, cigarette years, education level, p-years, and respondent. Confounders considered included marital status, smoking (age started smoking, average number of cigarettes, pipe or cigars smoked per day, total years smoked), and ethnicity.	Prostate cancer: OR 1.36 (0.83–2.25)	Conclusions: No specific conclusion given on glyphosate exposure and prostate cancer. JEM likely to result in non-differential misclassification and may underestimate the true association; thus, negative findings should be regarded as inconclusive. Limitations: Lack of information on familial history, potential for misclassification of exposure due to use of JEM, use of cancer controls may result in selection bias, statistically significant associations could have occurred by chance as a result of multiple comparisons since 142 active chemicals were examined.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Lee et al. 2004b Case control study of white men and women (ages ≥21 years) diagnosed with stomach adenocarcinoma (n=170) or esophagus adenocarcinoma (n=137) and 502 controls in eastern Nebraska to evaluate the risk of the stomach and esophageal adenocarcinomas associated with farming and agricultural use of 16 insecticides and 14 herbicides (including glyphosate). Glyphosate analysis included 12 cases of stomach cancer and 12 cases of esophageal cancer among farmers, and 46 controls compared to non-farmers (59 stomach cancer, 62 esophageal cancer cases and 184 controls). Controls were randomly selected from a group of controls interviewed in 1986–1987 for a previous population-based case-control study. Controls were frequency-matched by sex and age to the combined distribution of the stomach and esophagus cases.	Exposure: Self- or proxy-reported ever use of glyphosate pesticide at enrollment (1992–1994). Outcomes: Stomach and esophageal cancer cases were identified from the Nebraska Cancer Registry (1988–1990) or by review of discharge diagnosis and pathology records at 14 hospitals (1991–1993). Data analysis: Unconditional logistic regression. Adjustments: Age, sex. Confounders considered included BMI, smoking, alcohol consumption, educational level, family history of stomach or esophageal cancer, respondent type, dietary intake of vitamin A and C, b-cryptoxanthin, riboflavin, folate, zinc, dietary fiber, protein, and carbohydrate.	Stomach cancer: OR 0.8 (0.4–1.5) Esophageal cancer: OR 0.7 (0.3–1.4)	Conclusions: "No significant associations were found between specific agricultural pesticide exposures (including glyphosate) and the risk of stomach or esophageal adenocarcinomas among Nebraska farmers." Limitations: Possible misclassification of pesticide exposure and generally small number of farmers exposed to some of the individual pesticides.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Lee et al. 2005 Case control study of 251 white men and women (ages ≥21 years) diagnosed with gliomas and 498 controls in eastern Nebraska (Nebraska Health Study II) to evaluate adult glioma associated with farming and agricultural use of 20 insecticides and 17 herbicides (including glyphosate). Glyphosate analysis (only conducted among male farmers) included 17 cases and 32 controls among farmers compared to non-farmers (49 cases and 112 controls). Among these, self-reported respondents included 4 cases/17 controls for glyphosate users and 20 cases/40 controls for reference non-farmers; proxy-reported respondents included 13 cases/15 controls for glyphosate users and 29 cases/ 72 controls for reference non-farmers. Controls were randomly selected from a group of controls interviewed in 1986–1987 for a previous population-based case-control study. Controls were frequency-matched by sex, age, and vital status to the combined distribution of the cases.	Exposure: Self- or proxy-reported ever use of glyphosate pesticide at enrollment (1992–1994). Outcomes: Incident primary adult glioma cases diagnosed between 1988 and 1993 were identified from the Nebraska Cancer Registry or from 11 hospitals. Data analysis: Unconditional logistic regression. Separate analyses by sex and respondent type (self- versus proxy-reported) were also conducted. Adjustments: Age, sex, and respondent type. Confounders considered included history of head injury, marital status, education level, alcohol consumption, medical history of diabetes mellitus, dietary intake of a- and b-carotene, and dietary fiber.	Glioma among male farmers: OR 1.5 (0.7–3.1), all reported glyphosate use OR 0.4 (0.1–1.6), self-reported glyphosate use OR 3.1 (1.2–8.2), proxy-reported glyphosate use	Conclusions: "Glioma risk was also significantly increased among men who used specific pesticides (including glyphosate) and pesticide chemical classes; however, the positive results were mostly limited to proxy respondents." Limitations: The major limitation was the large proportion of proxy respondents. Most of the associations observed were limited to proxy respondents.

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Pahwa et al. 2011 Case control study of 357 soft tissue sarcoma cases and 1,506 controls in Canada (all males, ≥19 years of age) to investigate the putative associations of pesticides (including glyphosate) with soft-tissue sarcoma (STS). Glyphosate analysis included 36 exposed and 321 unexposed cases and 147 exposed and 1,359 unexposed controls. Potential controls were selected randomly within age constrains (±2 years) from provincial health records, comprehensive telephone lists, or voters' lists.	Exposure: Self-reported ever use of glyphosate herbicides collected through self-administered postal questionnaire and telephone interviews. Outcomes: STS cases (first diagnosed in 1991–1994) ascertained from provincial cancer registries, except in Quebec, where hospital ascertainment was used. Data analysis: Conditional logistic regression. Adjustments: Age, province of residence, medical history.	Soft tissue sarcoma: OR 0.93 (0.60–1.42), stratified by age group and province of residence OR 0.90 (0.58–1.40), adjusted for medical history and with strata for age group and province of residence	Conclusions: "No association between herbicides (individual compound or major chemical class) (including glyphosate) and STS." Limitations: Limitations common to epidemiological case-control studies.

142

Table 2-7. Cancer Outcomes for Solid Tumor-Types in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Yiin et al. 2012 Case control study of 798 cases of glioma and 1,175 controls (98% white, aged 18–80 years) in Iowa, Michigan, Minnesota, and Wisconsin (Upper Midwest Health Study) to investigate association between exposure to pesticides (including glyphosate) and risk of glioma in male and female participants. Pesticide use in non-farm jobs. Glyphosate analysis included 12 exposed and 786 unexposed cases and 147 exposed and 1,359 unexposed controls. Analysis included 8 exposed and 430 unexposed cases and 19 exposed and 1,122 unexposed controls excluding proxy respondents. House and garden pesticide use: Glyphosate analysis included 51 exposed and 747 unexposed cases and 76 exposed and 1,099 unexposed controls. Analysis included 28 exposed and 410 unexposed cases and 75 exposed and 1,066 unexposed controls excluding proxy respondents. Randomly selected, population-based controls were frequency-matched within a state.	Exposure: Self- or proxy-reported ever/never use of glyphosate pesticide through 1992. Outcomes: Cases with a histologically confirmed primary intracranial glioma were identified through medical facilities, oncologists, neurosurgeons, and cancer registries (1995–1997). Data analysis: Unconditional logistic regression. Analyses were separately conducted with or without proxy respondents. Adjustments: Age, sex, education.	Glioma Non-farm job use: OR 0.83 (0.39–1.73) including proxy respondents; OR 0.79 (0.33–1.86) excluding proxy respondents. House and garden use: OR 0.98 (0.67–1.43) including proxy respondents; OR 0.84 (0.52–1.33) excluding proxy respondents	Conclusions: "No individual pesticides (including glyphosate) or broader category of pesticides, with or without proxy respondent, was associated with a statistically significant decrease or elevation in glioma risk." Limitations: A limitation of this study is the high proportion (45%) of proxy interviews for case participants compared to 2.9% control interviews that were with proxies. The accuracy and completeness of information given by proxy respondents varies by many factors. Another concern is the validity and reliability of the pesticide exposure assessment.

BMI = body mass index; CED = cumulative exposure day; CI = confidence interval; IWED = intensity weighted exposure day; JEM = job exposure matrix; NHL = non-Hodgkin's lymphoma; OR = odds ratio; RR = relative risk; Q = quartile; STS = soft tissue sarcoma; T = tertile

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Andreotti et al. 2018 Prospective cohort study of 54,251 licensed pesticide applicators (97% white, 97% male) recruited between 1993 and 1997 in Iowa and North Carolina from the Agricultural Health Study to evaluate agricultural exposure to 50 pesticides (including glyphosate) and cancer incidence cases. 44,932 participants reported ever use of glyphosate, including 5,779 participants with incident cancer cases.	Exposure: Self-reported ever/never use of any glyphosate pesticides, lifetime days of glyphosate use (days per year x number of years), and intensity-weighted lifetime days (lifetime days x intensity score) at enrollment (1993–1997) or follow-up (1999–2005). Intensity-weighted lifetime days of glyphosate use was categorized into quartiles, tertiles, or the median, such that there were at least five exposed cases in each category. Outcome: Incident cancer diagnoses ascertained via linkage to cancer registries in Iowa (enrollment through 2013) and North Carolina (enrollment through 2012). Data analysis: Poisson regression Adjustments: Age, cigarette smoking status, alcohol drinks per month, family history of any cancer, state of recruitment, and the five pesticides (atrazine, alachlor, metolachlor, trifluralin, and 2,4-D). Confounders considered included BMI and pack-years of cigarettes smoked.	Lymphohematopoietic: Q4: RR 1.00 (0.74–1.34) p-trend: 0.43 Hodgkin's lymphoma: M2: RR 0.90 (0.25–3.24) p-trend: 0.94 NHL: Q4: RR 0.87 (0.64–1.20) p-trend: 0.95 B-cell: Q4: RR 0.86 (0.62–1.19) p-trend: 0.86 CLL/SLL: Q4: RR 0.87 (0.48–1.58) p-trend: 0.71 Diffuse large B-cell lymphoma: Q4: RR 0.97 (0.51–1.85) p-trend: 0.83 Marginal-zone lymphoma: M2: RR 0.44 (0.09–2.17) p-trend: 0.67 Follicular lymphoma: T3: RR 0.85 (0.36–2.03) p-trend: 0.95 Multiple myeloma: Q4: RR 0.87 (0.45–1.69) p-trend: 0.84	Conclusions: The authors observed no associations between glyphosate use and overall cancer risk or with total lymphohematopoietic cancers, including NHL, multiple myeloma, and any other NHL subtypes. There was some evidence of an increased risk of acute myeloid leukemia for applicators, particularly in the highest category of glyphosate exposure compared with never users of glyphosate. Risk estimates were similar in magnitude between the unlagged and lagged (5 or 20 years) exposure analyses for all sites evaluated. Limitations: Some misclassification of exposure undoubtedly occurred; because evaluated many cancer sites, cannot dismiss the possibility that results were observed by chance, and should be interpreted with caution; the fact that no other studies have reported an association with acute myeloid leukemia also calls for cautious interpretation.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
		NHL T-cell: M2: RR 1.53 (0.23–10.38) p-trend: 0.31 Acute myeloid leukemia: Q4: RR 2.44 (0.94–6.32) p-trend: 0.11 Chronic myeloid leukemia: M2: RR 0.82 (0.23–2.98) p-trend: 0.36	
De Roos et al. 2005a A prospective cohort study in 57,311 licensed pesticide applicators (>97% males) recruited between 1993 and 1997 in Iowa and North Carolina from the Agricultural Health Study to study cancer incidence associated with glyphosate use. All lymphohematopoetic: 190 (75.3%) NHL: 92 (77.2%) Leukemia: 57 (75.4) Multiple myeloma: 32 (75.0%)	Exposure: Self-reported never/ever use of glyphosate. Cumulative exposure days (CEDs): 1–20 (reference), 21–56, and 57–2,678 days. Intensity weighted exposure days (IWEDs) of 0.1–79.5 (reference), 79.6–337.1, and 337.2–18,241 units. Outcomes: Incident cases identified between enrollment and Dec 31st of 2001 from cancer registry files. Data analysis: Poisson regression adjusted for age, education, smoking status, alcohol consumption, family history of cancer in 1st degree relative, state of residence.	All lymphohematopoietic cancers: Ever use: RR 1.1 (0.8–1.6) CED T3: RR 1.2 (0.8–1.8) p-trend: 0.69 IWED T3: RR 1.0 (0.7–1.6) p-trend: 0.90 NHL cancers: Ever use: RR 1.1 (0.7–1.9) CED T3: RR 0.9 (0.5–1.8) p-trend: 0.73 IWED T3: RR 0.8 (0.5–1.4) p-trend: 0.99 Leukemia: Ever use: RR 1.0 (0.5–1.9) CED T3: RR 1.0 (0.4–2.9) p-trend: 0.61 IWED T3: RR 0.7 (0.2–2.1) p-trend: 0.11	Conclusions: Glyphosate exposure was not associated with overall cancer incidence or with most cancer subtypes, but there was a suggested association of glyphosate exposure with multiple myeloma incidence. Limitations: Small number of specific cancers cases, only males included in the analysis, no information on timing of pesticide use.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
		Multiple myeloma: Ever use: RR 2.6 (0.7–9.4) CED T3: RR 1.9 (0.6–6.3) p-trend: 0.27 IWED T3: RR 2.1 (0.6–7.0) p-trend: 0.17	
Sorahan 2015 Cohort study of 55,934 licensed pesticide applicators in Iowa and North Carolina (Agricultural Health Study). Set 1: 54,315 applicators, excluded those with cancer diagnosis before enrollment, those lost to follow-up, those who had missing data for age at enrollment, those who did not provide information on glyphosate use. ("Not known/missing" data included as a separate category for each variable.) n=32 cases. Set 2: 49,211 applicators, additionally excluded those with missing data on education, smoking history, or alcohol used. n=26 cases. Set 3: 40,719 applicators, additionally excluded those missing data on additional pesticide use. n=22 cases. Set 4: 55,934 applicators, excluding those with any cancer diagnosis prior to enrollment, those lost to follow up, and	Exposure: Self-reported never/ever use of glyphosate. CEDs: 1–20 (reference), 21–56, and 57–2,678 days. IWEDs of 0.1–79.5, 79.6–337.1, and 337.2–18,241 units. Outcomes: Incident cases identified between enrollment and December 31st from 2001 cancer registry files. Data analysis: Poisson regression adjusted for the following: Set 2: Age at enrollment, cigarette use, alcohol use, education. Set 4: Age at enrollment, cigarette use, alcohol use, education, family history of cancer. Sets 1 and 3: Age at enrollment, cigarette use, alcohol use, education, family history of cancer, use of some pesticides (2,4-D, alachlor, atrazine, metolachlor, trifluralin), ever use of other	Multiple myeloma: Set 1: Ever use: RR 1.24 (0.52–2.94) CED Q4: RR 1.38 (0.42–4.45) p-trend: 0.48 IWED Q4: RR 1.87 (0.67–5.27) p-trend: 0.22 Set 2: Ever use: RR 2.07 (0.71–6.04) Set 3: Ever use: RR 2.79 (0.78, 9.96) Set 4: Ever use: RR 1.18 (0.53–2.65) CED Q4: RR 1.17 (0.40–3.41) p-trend: >0.50 IWED Q4: RR 1.58 (0.62–4.05) p-trend: 0.30	Conclusions: Glyphosate is not a risk factor for multiple myeloma. Limitations: The small number of cases, absence of information on timing of pesticide exposure, unable to adjust for state of residence.

142

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
those missing data for age at enrollment. n=34 cases Re-analysis of data reported by De Roos et al. (2005a).	pesticides (maneb, paraquat, carbaryl, diazinon, benomyl).		
Brown et al. 1990 Case-control study of 578 cases of leukemia and 1,245 controls (all white males, ages ≥30 years) in Iowa and Minnesota to investigate agricultural exposure to 24 animal insecticides, 34 crop insecticides, 38 herbicides, and 16 fungicides (including glyphosate) and risk of leukemia. Glyphosate analysis included 15 cases and 49 controls who used glyphosate herbicide compared to never-farmers (243 cases and 547 controls). Controls were a population-based, stratified sample of white men frequency-matched to the cases by 5-year age group, vital status at interview, and state of residence.	Exposure: Self-reported ever mixing/handling/applying glyphosate herbicides at enrollment (1981–1984). Outcomes: Leukemia cases ascertained from Iowa Tumor Registry or hospital records in Minnesota from 1 year before (retrospectively) to 2 years after the start of the study (prospectively). Data analysis: Unconditional logistic regression. Adjustments: Vital status, age, state, tobacco use, family history of lymphopoietic cancer, high-risk non-farming occupations, high risk exposures (benzene, naphtha, hair dyes).	Leukemia OR 0.9 (0.5–1.6)	Conclusions: "Risks for all leukemia were not significantly increased among subjects who personally mixed, handled, or applied specific herbicides (including glyphosate)." Limitations: With the case-control study design, the associations found or failure to find an association could be due to bias. Potential inaccuracies in the evaluation of pesticide exposure could lead to exposure misclassification. Multiple statistical comparisons make it difficult to separate real association from chance findings.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Brown et al. 1993 Case control study to evaluate the association between multiple myeloma, agricultural risk factors, and exposure to individual pesticides in 823 white males aged ≥30 years in Iowa. 173 cases and 650 frequency-matched controls from random digit dialing, Medicare records, and death certificate files. Glyphosate analysis included 11 exposed and 162 unexposed cases (n=173) for multiple myeloma and 40 exposed and 610 unexposed controls (n=650).	Exposure: Self-reporting never/ever mixing, handling, or applying glyphosate. Outcomes: Multiple myeloma cases from the Iowa Health Registry from 1981 to 1984. Data analysis: Logistic models adjusted for vital status and age. Other confounders considered included smoking and education.	Multiple myeloma: OR 1.7 (0.8–3.6)	Conclusions: Little evidence of an association between risk of multiple myeloma and exposure to pesticides (including glyphosate). Limitations: Small number of cases and controls, multiple statistical comparisons, and possibility of recall bias or chance.
Cocco et al. 2013 Case control study of 4,810 in the EPILYMPH study from six European countries to investigate the role of occupational exposure to agrochemicals (including glyphosate) in etiology of lymphoma, B cell lymphoma and subtypes. 2,348 incident lymphoma cases and 2,462 controls (n=4,810). Glyphosate analysis included four exposed B cell lymphoma cases and two exposed controls.	Exposure: Self-reported questionnaires: never/ever glyphosate exposure. Outcomes: First diagnosis according to 2001 WHO classification of lymphoma between 1998 and 2004; patients referred from centers within referral area. Data analysis: Unconditional logistic regressions. Adjustments for age, gender, education, center.	B cell lymphoma: OR 3.1 (0.6–17.1)	Conclusions: No support to the role of occupation exposure to agrochemicals (including glyphosate) in etiology of B cell lymphoma. Limitations: Low response rate may have resulted in selection bias.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
De Roos et al. 2003 Pooled data from three case-control studies conducted by the National Cancer Institute to investigate exposure to multiple pesticides in farming as risk factors for NHL among 3,417 white males from Nebraska, Iowa, Minnesota, and Kansas. Glyphosate analysis included 36 exposed and 614 unexposed cases (n=650) and 61 exposed and 1,872 unexposed population based matched controls (n=1,933).	Exposure: Interview self-reported never/ever glyphosate exposure. Outcomes: In Nebraska, cases were identified through Nebraska Lymphoma Study Group and area hospitals among males aged ≥21 years from July 1983 to June 1986. In Iowa, cases were ascertained from Iowa State Health Registry from 1981 to 1983 from males ≥30 years of age. In Minnesota, cases were ascertained from a surveillance system of Minnesota hospitals and pathology laboratories from 1980 to 1982 in males ≥30 years of age. In Kansas, cases were randomly selected from statewide cancer registry from males ≥21 years of age. Data analysis: Two models were used: (1) standard logistic regression and (2) hierarchical regression adjusted for age and study site.	Logistic regression: NHL: OR 2.1 (1.1–4.0) Hierarchical regression: NHL: OR 1.6 (0.9–2.8)	Conclusions: No specific conclusions for glyphosate and NHL. Limitations: Crude exposure metric, no information on timing of exposure versus NHL onset or timing of use of pesticides to each other. Potential bias for missing data exclusion.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Eriksson et al. 2008 Case control study of 1,926 male and female subjects aged 18–74 years were recruited between December 1, 1999 and April 30, 2002 in Sweden to evaluate pesticides (including glyphosate) as a risk factor for NHL. Glyphosate analysis included 29 exposed and 881 unexposed cases (n=910) and 18 exposed and 998 unexposed frequency-match controls (n=1,016).	Exposure: Self-reporting questionnaires; never/ever exposed and days of exposure. Outcomes: Newly diagnosed NHL, identified through physicians and pathologists recruited between December 1, 1999 and April 30, 2002. Subtypes divided according to WHO classification. Data analysis: Unconditional logistic regression analysis adjusted for age, sex, year of diagnosis/enrollment.	NHL: Ever: OR 2.02 (1.10–3.71) Ever (adjusted for other pesticides): OR 1.51 (0.77–2.94) Ever (1–10-year latency): OR 1.11 (0.24–5.08) Ever (>10-year latency): OR 2.26 (1.16–4.40) ≤10 days: OR 1.69 (0.70–4.07) ≥10 days: OR 2.36 (1.04–5.37) B-cell lymphomas: Ever: OR 1.87 (0.998–3.51) Lymphocytic lymphoma: Ever: OR 3.35 (1.42–7.89) Follicular, grade I-III: Ever: OR 1.89 (0.62–5.79) Diffuse large B-cell lymphoma: Ever: OR 1.22 (0.44–3.35) Other specified B-cell lymphoma: Ever: OR 1.63 (0.53–4.96) Unspecified B-cell lymphoma: Ever: OR 1.47 (0.33–6.61) T-cell lymphoma: Ever: OR 2.29 (0.51–10.4) Unspecified NHL: Ever: OR 5.63 (1.44–22.0)	Conclusions: The association of NHL with glyphosate was strengthened by the study. Limitations: No registries of pesticide use kept in Sweden, possible misclassification of pesticide exposure, no information gathered on protective equipment use.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Hardell et al. 2002 Pooled analysis of two case-control studies of 1,656 male and female subjects from Sweden to investigate pesticides in etiology of NHL and HCL. Glyphosate analysis included 8 exposed and 507 unexposed cases (n=515) and 8 exposed and 1,133 unexposed county-matched controls (n=1,141).	Exposure: Self-reporting questionnaires; never/ever glyphosate exposure. Outcomes: Histopathologically verified NHL cases from regional cancer registries in males age ≥25 years from 1987 to 1990. HCL diagnosed cases from the national Swedish Cancer Registry in males from 1987 to 1992. Data analysis: Conditional logistic regression analysis adjusted for both univariate and multivariate.	NHL and HCL (pooled): Ever (univariate analysis): OR 3.04 (1.08–8.52) Ever (multivariate analysis): OR 1.85 (0.55–6.20)	Conclusions: Glyphosate is a risk factor for developing NHL. Limitations: Possible recall bias. Correlation of pesticides.
Kachuri et al. 2013 A population-based, case-control study in 1,506 males from six Canadian provinces to investigate the association between lifetime use of multiple pesticides and multiple myeloma. Glyphosate analysis included 32 exposed cases and 310 unexposed cases (n=342) and 121 exposed and 1,236 unexposed frequency-matched controls (n=1,357). Excluding proxy respondents, analysis included 23 exposed cases and 108 exposed frequency-matched controls.	Exposure: Self-reporting questionnaires; ever/never, days/year glyphosate use. Outcomes: Incident multiple myeloma cases among men aged ≥19 years who were diagnosed between September 1, 1991 and December 31, 1994 ascertained from provincial cancer registries. Cases in Quebec were ascertained from hospitals. Data analysis: Logistic regression. Adjusted for age, province of residence, use of proxy responders, smoking, and selected medical history.	Multiple myeloma: Ever: OR 1.19 (0.76–1.87) Ever (exclude proxies): OR 1.11 (0.66–1.86) >0 and ≤2 days/year: OR 0.72 (0.39–1.32) >0 and ≤2 days/year (exclude proxies): OR 0.70 (0.35–1.40) >2 days/year: OR 2.04 (0.98–4.23) >2 days/year (exclude proxies): OR 2.11 (0.95–4.70)	Conclusions: No specific conclusions for glyphosate and NHL. Limitations: Low response rates observed for cases and controls, possibility of recall bias.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Karunanayake et al. 2012 Case-control study of 1,822 men to evaluate exposure to pesticides and incidence of Hodgkin lymphoma in six Canadian provinces. Glyphosate analysis included 38 exposed and 278 unexposed Hodgkin lymphoma cases (n=316) and 133 exposed and 1,373 unexposed age-matched controls (n=1,506).	Exposure: Any self-reported glyphosate use. Outcomes: Hodgkin lymphoma incidences determined using Internal Classification of Diseases for Oncology, 2nd Edition (ICD-O-2) from September 1, 1991 to December 31, 1994. Data analysis: Conditional logistic regression. Adjustments for age, province of residence, personal and family medical history.	Hodgkin lymphoma: OR 0.99 (0.62–1.56)	Conclusions: This study shows a lack of association between Hodgkin lymphoma and glyphosate. Limitations: Inability to ascertain Epstein-Barr virus exposure. Potential for recall bias and for misclassification of exposure to pesticides, as well as misclassification of exposure duration. Low response rates resulted in inability to evaluate dose-response relationship and women were not included in the study.
Lee et al. 2004a Case control study of 3,253 in Iowa, Minnesota, and Nebraska to evaluate if asthma modifies risk associated with pesticide exposure. 872 cases of NHL and 2,381 frequency-matched controls. Glyphosate analyses, 259 cases and 684 controls for non-asthmatic non-farmers (reference), 53 cases and 91 controls for non-asthmatic farmers, and 6 cases and 12 controls for asthmatic farmers. These data were used in the pooled analysis by De Roos et al. (2003).	Exposure: Self-reported ever/never glyphosate use. Self-reported asthma from physician diagnosis. Outcomes: Cases identified through Iowa State Health Registry and Minnesota's surveillance system of hospital and pathology laboratories from 1980 to 1983 (n=530). Cases identified through Nebraska Lymphoma Study group and area hospitals between July 1983 and June 1986 (n=346). Data analysis: Unconditional logistic regression adjusted for age, state, vital status.	NHL(non-asthmatic farmers): OR 1.4 (0.98–2.1) NHL (asthmatic farmers): OR 1.2 (0.4–3.3)	Conclusions: No specific conclusion concerning exposure to glyphosate, asthma, and NHL. Limitations: Self-reported exposure and asthma diagnosis may be subject to misclassification bias.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
McDuffie et al. 2001 Case-control study to investigate the association between non-occupational exposure to pesticides (including glyphosate) and NHL among 2,023 men in six Canadian provinces. Glyphosate analysis included 51 exposed and 466 unexposed NHL cases (n=517) and 133 exposed and 1,373 unexposed age-matched controls (n=1,506).	Exposure: Self-reported ever/never use of any glyphosate use and number days/year use. Outcomes: First diagnosis of NHL between September 1, 1991 and December 31, 1991 from cancer registries for five providences, in Quebec where hospital records were used. Data analysis: Conditional logistic regression adjusted for age, province of residence, medical history (measles, mumps, cancer, allergy desensitization shots, positive family history of cancer in 1st-degree relative).	NHL: Ever use: OR 1.20 (0.83–1.74) Exposure >0 and ≤2 days/year: OR 1.00 (0.63–1.57) Exposure >2 days/year: OR 2.12 (1.20–3.73)	Conclusions: No conclusions stated for glyphosate ever use. When stratified by average number of days per year of exposure, glyphosate was not significant for exposure, but demonstrated a dose-response relationship. Limitations: Potential for recall bias and misclassification of pesticide exposure. Inclusion of occupational groups without extensive validations studies could bias findings towards null. Less-than-optimal response rates. Due to multiple comparison, a small number of statistically significant results may be attributable to chance. Because of limited statistical power, analysis was restricted to exposure that at least 1% of respondents ever used.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Nordström et al. 1998 Case-control study of 511 Swedish adult males to evaluate occupational exposures (including glyphosate) as risk factors for HCL. Glyphosate analysis included 4 exposed and 107 unexposed cases (n=111) of HCL and 5 exposed and 395 controls (n=400) in Sweden. These data were used in pooled analysis by De Roos et al. (2003).	Exposure: Self-reported never/ever glyphosate exposure determined by at least 1 working day (8 hours) and induction of at least 1 year. Outcomes: HCL reported to Swedish Cancer Registry from 1987 to 1992. One case diagnosed in 1993 included in analysis. Data analysis: Logistic regression adjusted for age.	HCL: OR 3.1 (0.8–12)	Conclusions: No specific conclusions were given for glyphosate. Limitations: Possible correlation of occupational exposures resulting in confounding. Multiple comparisons may result in some correlations to occur by chance. Possibility of elevated OR due to recall bias.

Table 2-8. Lymphohematopoietic Cancer Outcomes in Humans Exposed to Glyphosate-Containing Products

Reference and study overview	Methods and outcomes	Results (with 95% CI)	Study author conclusions and limitations
Orsi et al. 2009 Case-control study to investigate the relationship between occupational exposure to pesticides and lymphoid neoplasms in 947 18–75-year-old males from six hospitals in France from 2000 to 2004. Glyphosate analysis included: 12 exposed and 232 unexposed NHL cases (n=244) and 24 exposed and 412 unexposed center, age, sex-matched controls (n=436). 6 exposed and 81 unexposed cases of Hodgkin's lymphoma (n=87) and 15 exposed and 250 unexposed center, age, sex-matched controls (n=265). 5 exposed and 51 unexposed cases of multiple myeloma (n=56) and 18 exposed and 295 unexposed center, age, sex-matched controls (n=313). 27 exposed and 464 unexposed cases of lymphoid neoplasms (n=491) and 24 exposed and 432 unexposed center, age, sex-matched controls (n=456).	Exposure: Self-reported none and probable/definite glyphosate exposure, after expert review of pesticide use questionnaire. Outcomes: Cases determined using ICD-O-3 code diagnosis from September 2000 to December 2004. Data analysis: Unconditional logistic regression, adjusted for age, center, socioeconomic category (white collar/blue collar).	Lymphoid neoplasms: OR 1.2 (0.6–2.1) NHL: OR 1.0 (0.5–2.2), all subtypes OR 1.0 (0.3–2.7) for diffuse large cell lymphoma OR 1.4 (0.4–5.2) for follicular lymphoma Hodgkin's lymphoma: OR 1.7 (0.6–5.0) Lymphoproliferative syndrome: OR 0.6 (0.2–2.1), all subtypes OR 0.4 (0.1–1.8) for chronic lymphocytic leukemia OR 1.8 (0.3–9.3) for HCL Multiple myeloma: OR 2.4 (0.8–7.3)	Conclusions: No specific conclusions for glyphosate. Limitations: Potential non-differential misclassification resulting in reduced power.

an association (OR 1.6; 95% CI 0.9–2.8). Similarly, Eriksson et al. (2008) reported a positive association with NHL (OR 2.02; 95% CI 1.10–3.71); when this analysis further adjusted for other pesticide use, the reported OR was 1.51 (95% CI 0.7–2.94). Hardell et al. (2002) investigated the association between glyphosate use and combined cases of NHL and hairy cell leukemia. The authors reported an OR of 3.04 (95% CI 1.08–8.52) in unadjusted models, but after adjusting for potential confounders, the reported OR was 1.85 (95% CI 0.55–6.20). McDuffie et al. (2001) reported that glyphosate use was not associated with NHL (OR 1.20; 95% CI 0.83–1.74); however, after restricting analyses to individuals who reported using glyphosate >2 days a year, there was a positive association with NHL (OR 2.12; 95% CI 1.20–3.73).

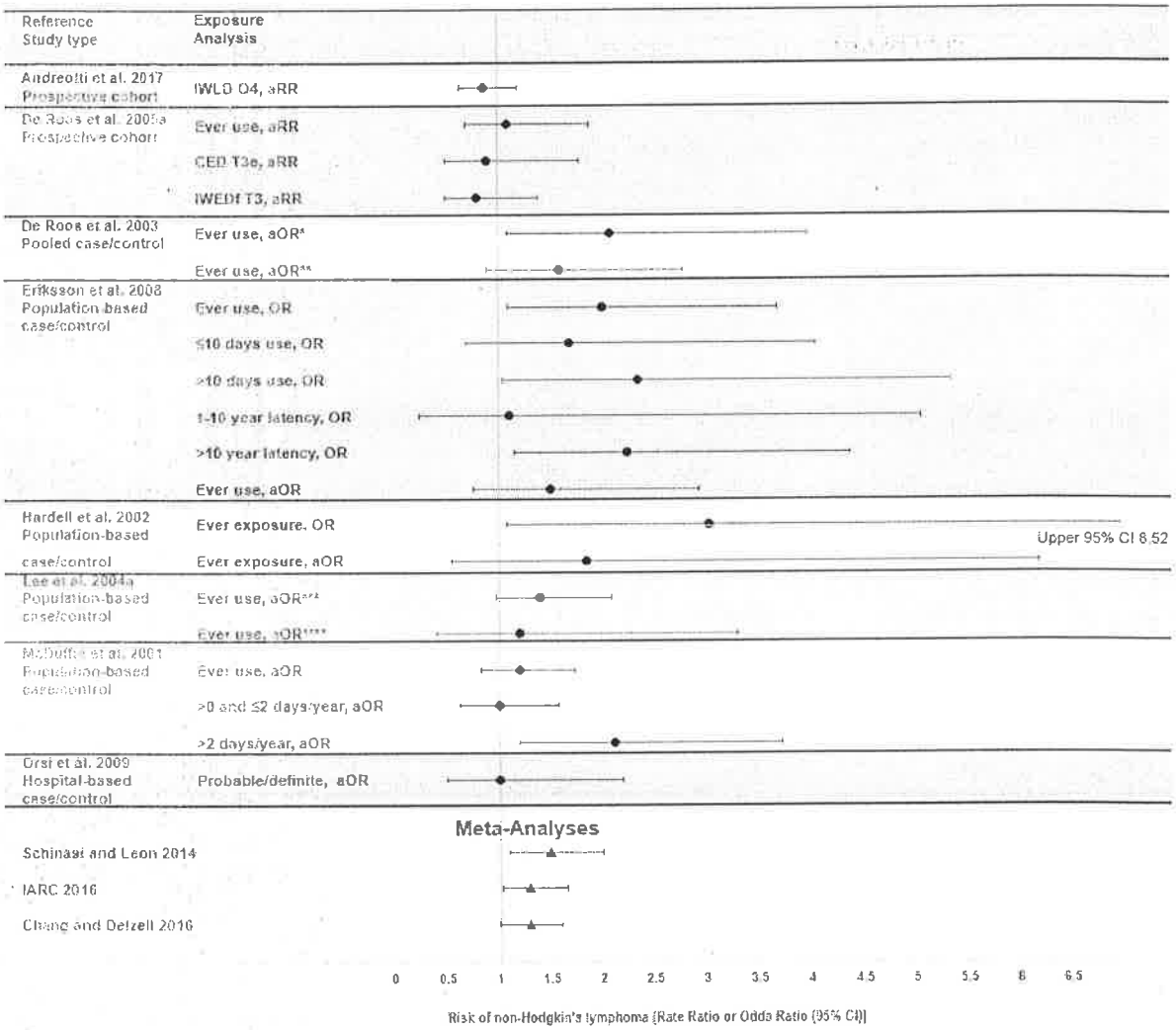
Results for risk of non-Hodgkin's lymphoma and self-reported glyphosate use or exposure from individual studies summarized in Table 2-8 and meta-analyses summarized in Table 2-6 are plotted in Figure 2-4. Results for risk of multiple myeloma and self-reported glyphosate use or exposure from individual studies summarized in Table 2-8 and the meta-analysis summarized in Table 2-6 are plotted in Figure 2-5.

Laboratory Animal Studies

EPA evaluated results from four unpublished rat studies in which the carcinogenicity of glyphosate technical was assessed; EPA summarized the findings in publicly-available DERs (EPA 1991a, 1991b, 1992d, 2015c).

Groups of weanling Sprague-Dawley rats (50/sex/group) were administered glyphosate technical (98.7% purity) in the diet for up to 26 months at initial concentrations of 0, 30, 100, or 300 ppm (EPA 1992d). Based on body weight and food consumption data, concentrations of glyphosate technical were adjusted to achieve oral doses of 0, 3.05, 10.30, and 31.49 mg/kg/day, respectively, for males and 0, 3.37, 11.22, and 34.02 mg/kg/day, respectively, for females. Incidences of testicular interstitial cell tumors in the control, low-, mid-, and high-dose male rats were 0/50 (0%), 3/50 (6%), 1/50 (2%), and 6/50 (12%), respectively (Table 2-9). The incidence in the high-dose males was statistically significant ($p=0.013$) in pairwise comparison to the control incidence. Although the incidence in the mid-dose group was less than that in the low-dose group, trend analysis revealed a significant trend ($p=0.009$) for increasing incidence of testicular interstitial cell tumors with increasing dose. Evaluation of historical control incidences resulted in testicular interstitial cell tumor incidences in the range of 0–12%, with a mean incidence of 4.5% (range: 3.4–6.7%) among lifetime studies that employed the same rat strain and were conducted concurrently with the 26-month study.

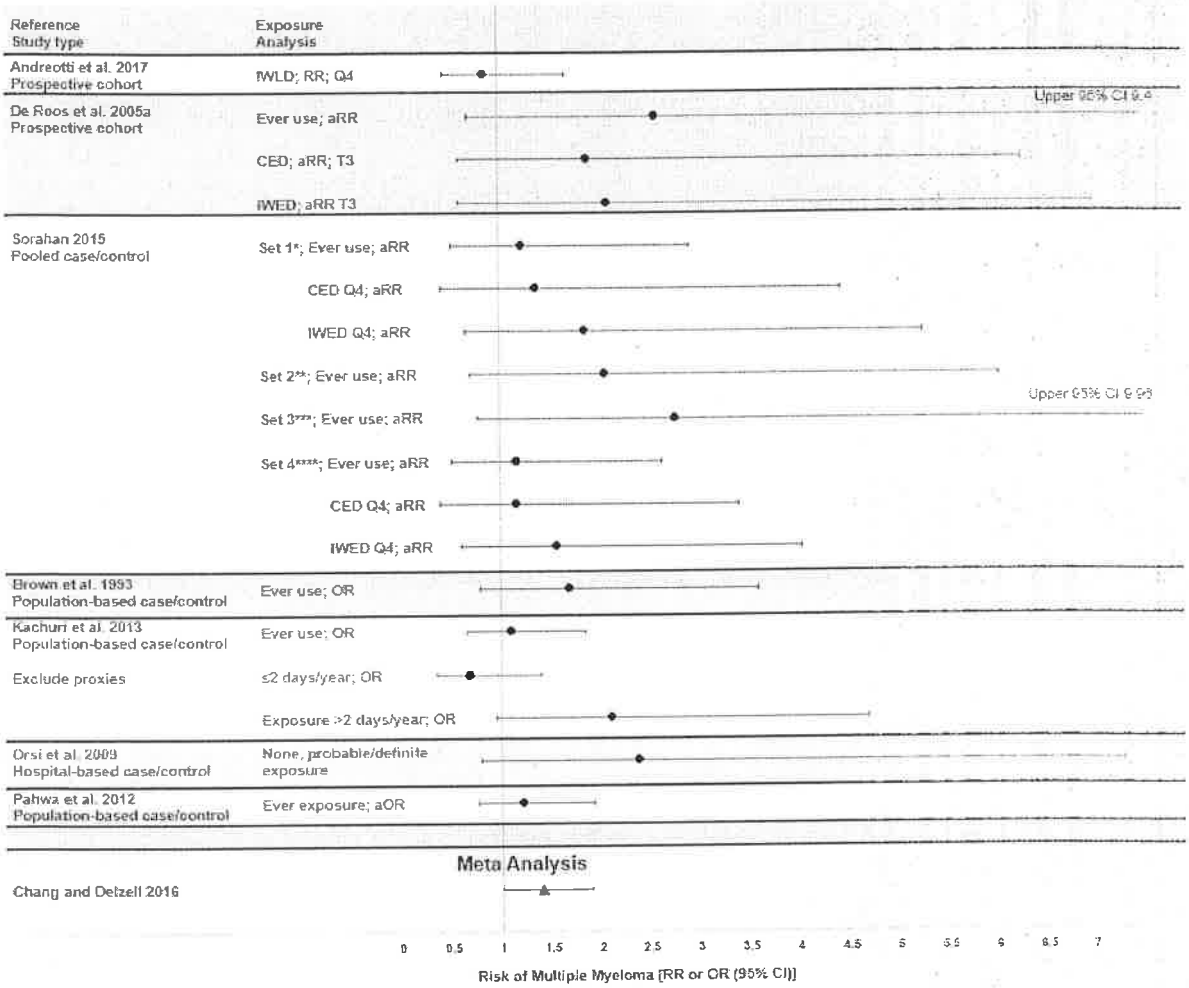
Figure 2-4. Risk of non-Hodgkin's Lymphoma Relative to Self-Reported Glyphosate Use or Exposure



*Logistic Regression; **Hierarchical regression; ***Non-Asthmatic farmers; ****Asthmatic farmers

a = adjusted; CED = cumulative exposure; IWED = intensity-weighted exposure days; IWLD = intensity-weighted lifetime days; OR = odds ratio; Q4 = 4th quartile; RR = rate ratio; T3 = 3rd tertile

Figure 2-5. Risk of Multiple Myeloma Relative to Self-Reported Glyphosate Use or Exposure



*Set 1 included 54,315 applicators; **Set 2 included 49,211 applicators; ***Set 3 included 40,719 applicators; ****Set 4 included 55,934 applicators

a = adjusted; CED = cumulative exposure; IWED = intensity-weighted exposure days; IWLD = intensity-weighted lifetime days; IRED = intensity-rated exposure days; OR = odds ratio; Q4 = 4th quartile; RR = rate ratio; T3 = 3rd tertile

Incidences of thyroid c-cell tumors (adenoma, carcinoma, combined adenoma or carcinoma) in the female rats are presented in Table 2-9. An increased incidence of thyroid c-cell carcinomas in female rats approached statistical significance (p=0.055) at the highest dose (6/47 versus 1/47 for controls) (EPA 1992d). The combined incidence of combined c-cell carcinomas or adenomas was not significantly increased (9/47 high-dose females versus 6/47 controls), and time-to-tumor analysis revealed no sign of a treatment-related effect. Historical control incidences of spontaneous thyroid c-cell tumors in female Sprague-Dawley rats were as high as 17%.

Table 2-9. Incidences of Selected Tumors in Sprague-Dawley Rats Administered Technical Glyphosate (98.7% purity) in the Diet for up to 26 Months

	Glyphosate dose (mg/kg/day)				Historical control incidence
	0	3.05	10.3	31.49	
Male rats					
Testes interstitial cell tumors					
Interstitial cell tumors	0/50 (0%)	3/50 (6%)	1/50 (2%)	6/50[a] (12%)	0–12%
Female rats					
Thyroid c-cell tumors					
Adenoma	5/47 (11%)	3/49 (6%)	6/50 (14%)	3/47 (6%)	0–17%
Carcinoma	1/47 (2%)	0/49 (0%)	2/50 (4%)	6/47 (13%)	0–5%
Adenoma or carcinoma (combined)	6/47 (13%)	3/49 (6%)	8/50 (16%)	9/47 (19%)	0–17%

[a]Significantly different from concurrent control according to Fisher's Exact Test (p<0.05).

NA = not applicable; NS = not specified

Sources: EPA 1992d

Groups of albino Sprague-Dawley rats (60/sex/group) were administered technical glyphosate (96.5% purity) in the diet at target concentrations of 0, 2,000, 8,000, or 20,000 ppm (mean measured concentrations of 0, 1,900, 7,600, and 19,000 ppm, respectively) for up to 24 months (EPA 1991a, 1991b). Based on mean body weight and food consumption data, estimated glyphosate doses to controls and low-, mid-, and high-dose groups were 0, 89, 362, and 940 mg/kg/day, respectively, for the males and 0, 113, 457, and 1,183 mg/kg/day, respectively, for the females.

As shown in Table 2-10, low-dose (but not mid- or high-dose) males exhibited significantly increased incidences of pancreatic islet cell adenoma (p=0.015) in pairwise comparison to control incidence (EPA 1991a, 1991b). Incidences of pancreatic islet cell carcinoma in low-, mid-, and high-dose males were not significantly different from control incidences. Incidences of combined adenoma or carcinoma among

mid-, and high-dose males were not significantly different from control incidences. After excluding those male rats that died or were sacrificed prior to treatment week 55 (before the first adenoma or carcinoma were observed), incidences of pancreatic islet cell adenoma in the low-dose group remained significantly (p=0.018) higher than controls. However, exclusion of the early deaths resulted in only borderline significantly increased incidence of combined adenoma or carcinoma (p=0.052) in the low-dose group. Historical control incidences for pancreatic islet cell adenoma in male rats from 2-year studies conducted at the same testing facility ranged from 1.8 to 8.5%. In the female rats, no significant differences were observed between controls and treated rats regarding pancreatic islet cell tumor incidences in pairwise comparisons with controls.

Table 2-10. Incidences of Selected Tumors in Albino Sprague-Dawley Rats Administered Technical Glyphosate (96.5% Purity) in the Diet for 2 Years

	Glyphosate dose (mg/kg/day)				Historical control incidence
	0	89	362	940	
Male rats					
Pancreatic islet cell tumors					
All deaths considered					
Adenoma	1/58 (2%)	8/57[a] (14%)	5/60 (8%)	7/59 (12%)	1.8–8.5%
Carcinoma	1/58 (2%)	0/57 (0%)	0/60 (0%)	0/59 (0%)	NS
Adenoma or carcinoma (combined)	2/58 (3%)	8/57 (14%)	5/60 (8%)	7/59 (12%)	NA
Excluding deaths prior to treatment week 55 (first adenoma at week 81; first carcinoma at week 105)					
Adenoma	1/43 (2%)	8/45[a] (18%)	5/49 (8%)	7/48[a] (15%)	NA
Carcinoma	1/43 (2%)	0/45 (0%)	0/49 (0%)	0/48 (0%)	NA
Adenoma or carcinoma (combined)	2/43 (2%)	8/45 (18%)	5/49 (10%)	7/48 (15%)	NA
Thyroid c-cell tumors					
All deaths considered					
Adenoma	2/60 (3%)	4/58 (7%)	8/58[b] (14%)	7/60 (12%)	1.8–10.6%
Carcinoma	0/60 (0%)	2/58 (3%)	0/58 (0%)	1/60 (2%)	NS
Excluding deaths prior to treatment week 55 (first adenoma at week 54; first carcinoma at week 93)					
Adenoma	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)	NA
Carcinoma	0/54 (0%)	2/55 (4%)	0/58 (0%)	1/58 (1%)	NA
Adenoma or carcinoma (combined)	2/54 (4%)	6/55 (11%)	8/58 (14%)	8/58 (14%)	NA
Liver tumors					
All deaths considered					
Adenoma	2/60 (3%)	2/60 (3%)	3/60 (5%)	7/60 (12%)	1.4–18.3%
Carcinoma	3/60 (5%)	2/60 (3%)	1/60 (2%)	2/60 (3%)	0–6.7%

Table 2-10. Incidences of Selected Tumors in Albino Sprague-Dawley Rats Administered Technical Glyphosate (96.5% Purity) in the Diet for 2 Years

	Glyphosate dose (mg/kg/day)				Historical control incidence
	0	89	362	940	
Excluding deaths prior to treatment week 55 (first adenoma at week 88; first carcinoma at week 85)					
Adenoma	2/44 (5%)	2/45 (4%)	3/49 (6%)	7/48 (15%)	NA
Carcinoma	3/44 (7%)	2/45 (4%)	1/49 (2%)	2/48 (4%)	NA
Adenoma or carcinoma (combined)	5/44 (11%)	4/45 (9%)	4/49 (8%)	9/48 (19%)	NA
Female rats					
Pancreatic islet cell tumors					
All deaths considered					
Adenoma	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)	NS
Carcinoma	0/60 (0%)	0/60 (0%)	0/60 (0%)	0/59 (0%)	NS
Adenoma or carcinoma (combined)	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)	NA
Thyroid c-cell tumors					
All deaths considered					
Adenoma	2/60 (3%)	2/60 (3%)	6/60 (10%)	7/60 (10%)	3.3–10%
Carcinoma	0/60 (0%)	0/60 (0%)	1/60 (2%)	0/60 (0%)	0–2.9%
Adenoma or carcinoma (combined)					
Excluding deaths prior to treatment week 55 (first adenoma at week 72; first carcinoma at week 93)					
Adenoma	2/57[c] (4%)	2/60 (3%)	6/59 (10%)	6/55 (11%)	NS
Carcinoma	0/57 (0%)	0/60 (0%)	1/59 (2%)	0/55 (0%)	NS
Adenoma or carcinoma (combined)	2/57[c] (4%)	2/60 (3%)	7/59 (12%)	6/55 (11%)	NA

[a]Significantly different from concurrent control according to Fisher's Exact Test ($p<0.05$).
[b]Marginally significantly different from concurrent control according to Fisher's Exact Test ($p=0.051$).
[c]Significant trend ($p<0.05$) for increasing incidence of adenoma and adenoma/carcinoma combined, excluding deaths prior to treatment week 55.

NA = not applicable; NS = not specified

Sources: EPA 1991a, 1991b

As shown in Table 2-10, the incidence of thyroid c-cell adenoma in mid-dose (but not low- or high-dose) male rats was marginally significantly ($p=0.051$) greater than that of controls. Historical control incidences for thyroid c-cell adenoma in male rats ranged from 1.8 to 10.6%. Pairwise comparison with concurrent controls revealed no significant difference between controls and low-, mid-, or high-dose groups regarding incidences of thyroid c-cell adenoma or carcinoma. There were no significant differences between controls and low-, mid-, or high-dose groups regarding incidences of thyroid c-cell adenoma after excluding those male rats that died prior to week 54 (EPA 1991a, 1991b). In the female

rats, no significant differences were observed between controls and treated rats regarding thyroid c-cell tumor incidences in pairwise comparisons with controls. Significant trends ($p<0.05$) for increasing incidence of adenoma and adenoma/carcinoma combined were noted after excluding those female rats that died prior to week 55 (EPA 1991a, 1991b).

As shown in Table 2-10, incidences of liver tumors in the glyphosate-treated male rats were not significantly different from incidences among controls. Lack of statistical significance remained after excluding those rats that died or were sacrificed prior to study week 55 and upon combining incidences of adenoma or carcinoma combined.

EPA summarized results from two unpublished rat studies in which the carcinogenicity of glyphosate technical was assessed. In one study, groups of Alpk:APfSD Wistar rats (64/sex/group) received glyphosate (97.6% purity) from the diet for up to 2 years at 0, 121, 361, or 1,214 mg/kg/day (males) and 0, 145, 437, or 1,498 mg/kg/day (females) (EPA 2015c). An interim sacrifice was performed on 12 rats/sex/group after 1 year. Incidences of hepatocellular adenoma among controls, low-, mid-, and high-dose male rats were reported as 0/52 (0%), 2/52 (4%), 0/52 (0%), and 5/52 (10%), respectively. The incidence in the high-dose group was significantly greater than that of controls ($p=0.028$ by Fisher's exact test). EPA (2015c) noted a range of 0–11.5% for this tumor type among historical controls reported by Greim et al. (2015). In the other study, there were no treatment-related increased incidences of any tumor type among Sprague-Dawley rats (50/sex/group) that received glyphosate (98.9 purity) from the diet for up to 104 weeks at 0, 100, 300, or 1,000 mg/kg/day (EPA 2015c).

In a combined chronic toxicity/carcinogenicity study, groups of Sprague-Dawley rats (50/sex/group for the carcinogenicity portion) received glyphosate (98.9 purity) from the diet for up to 104 weeks at 0, 100, 300, or 1,000 mg/kg/day (EPA 2015c). There were no treatment-related increased incidences of any tumor type.

EPA also evaluated results from two unpublished mouse studies in which the carcinogenicity of glyphosate technical was assessed; EPA summarized the findings in publicly-available DERs.

In one study, groups of CD-1 mice (50/sex/group) were administered technical glyphosate (99.78% purity) for 24 months at doses of 0, 161, 835, or 4,945 mg/kg/day to the males and 0, 195, 968, or 6,069 mg/kg/day to the females (EPA 2015a; selected results also available in EPA 1985a, 1985b, 1986b, 1989, and 1993). Guidelines for testing of chemicals for carcinogenicity generally consider

1,000 mg/kg/day as an upper limit for oral dosing (e.g., OECD Test Guideline 451, available at: http://www.oecd.org/chemicalsafety/testing/41753121.pdf). The highest dose tested in the mouse study far exceeds the upper limit and the mid-dose level approached the upper limit. There were no treatment-related effects on tumor incidences in the female mice. Table 2-11 shows incidence data for renal tubular cell tumors in the male mice summarized by EPA (2015a). There were no statistically significant trends for increased incidence of renal tubule adenoma, carcinoma, or combined carcinoma or adenoma and no statistically significant differences between groups upon pairwise analyses.

Table 2-11. Incidences of Renal Tubular Cell Tumors in Male CD-1 Mice Administered Technical Glyphosate (99.78% Purity) in the Diet for up to 24 Months

	Dose (mg/kg/day)			
	0	161	835	4,945
Adenoma	1/49 (2%)	0/49 (0%)	0/50 (0%)	1/50 (2%)
Carcinoma	0/49 (0%)	0/49 (0%)	1/50 (2%)	2/50 (4%)
Adenoma or carcinoma (combined)	1/49 (2%)	0/49 (0%)	1/50 (2%)	3/50 (6%)

Source: EPA 2015a

In the other study, groups of CD-1 mice (50/sex/group) received glyphosate (≥97.5% purity) from the diet at 0, 100, 300, or 1,000 mg/kg/day for 104 weeks (EPA 2015c). Incidence data for tumors reported by EPA are summarized in Table 2-12. Compared to controls, the incidence of hemangiosarcoma in the high-dose males approached the level of statistical significance (p=0.056 according to Fishers exact test). A significant trend (p=0.00296) was noted for increased incidence of hemangiosarcoma with increasing dose. All tumors were malignant and were located in the liver and spleen of one mouse; liver of another mouse; spleen of a third mouse; and liver, spleen, and prostate of the fourth mouse. Hemangiosarcoma incidences among glyphosate-treated female mice were not significantly increased relative to controls. All tumors were malignant and were located in the uterus of one low-dose female, spleen of another low-dose female, and liver of the high-dose female.

Table 2-12. Incidences of Tumors in Male and Female CD-1 Mice Administered Glyphosate (≥97.5% Purity) in the Diet for up to 104 Weeks

	Dose (mg/kg/day)			
	0	100	300	1,000
	Males			
Hemangiosarcoma	0/50[a] (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
Histiocytic sarcoma	0/50 (0%)	2/50 (4%)	0/50 (0%)	2/50 (4%)
	Females			
Hemangiosarcoma	0/50 (0%)	2/50 (4%)	0/50 (0%)	1/50 (2%)
Histiocytic sarcoma	0/50 (0%)	3/50 (6%)	3/50 (6%)	1/50 (2%)

[a]Significant trend (p=0.00296) for increasing incidence of hemangiosarcoma

Source: EPA 2015c

George et al. (2010) evaluated the potential carcinogenicity of Roundup Original® using the 2-stage mouse skin carcinogenesis model. The study included groups of male Swiss albino mice (20/group) receiving the glyphosate formulation topically 3 days/week for 32 weeks, single topical application of dimethylbenz[a]anthracene (DMBA; a tumor initiator) followed by repeated dermal applications of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA; a tumor promoter), single or multiple topical application of the glyphosate formulation followed by dermal applications of TPA (test for initiation potential of the glyphosate formulation), single application of DMBA followed by repeated dermal application of the glyphosate formulation (test for promotion potential of the glyphosate formulation), single DMBA application, repeated TPA application, and untreated controls. Skin tumors were observed in 100% of the DMBA + TPA treatment group; the first tumor appeared at 52 days. Tumors were noted in 40% of the DMBA + glyphosate formulation treatment group; the first tumor appeared at 130 days. No tumors were observed in other groups. The results indicate that the glyphosate formulation functioned as a tumor promoter, but not a tumor initiator or complete carcinogen.

Assessment of Carcinogenicity. Several national and international agencies and organizations have assessed the carcinogenicity of glyphosate (Table 2-13). These evaluations provide different types of determinations—some focused on hazard identification, or whether there is evidence that a chemical can cause an effect, and others focused on carcinogenic risk, or the likelihood of cancer effects at levels of exposure typically experienced by humans. In addition, there are large numbers of unpublished guideline studies on glyphosate and the inclusion or exclusion of these may account for the differences in the conclusions reached by these various agencies. For additional discussion regarding the carcinogenicity of

Table 2-13. Carcinogenicity Classification

Organization	Reference	Classification	Justification
Domestic organizations			
U.S. Environmental Protection Agency	EPA 2017c	Strongest support is for "not likely to be carcinogenic to humans".	According to 2005 Guidelines for Carcinogen Risk Assessment, EPA (2005a), considered that the strongest support for a carcinogenicity classification for glyphosate is the descriptor "not likely to be carcinogenic to humans." EPA (2017c) concluded "there is not strong support for the 'suggestive evidence of carcinogenic potential' cancer classification descriptor based on the weight-of-evidence, which includes the fact that even small, non-statistically significant changes observed in animal carcinogenicity and epidemiological studies were contradicted by studies of equal or higher quality."
International organizations			
Australian Pesticides and Veterinary Medicines Authority	APVMA 2017	Exposure does not pose a carcinogenic risk to humans	Concluded "that the scientific weight-of-evidence indicates that exposure to glyphosate does not pose a carcinogenic risk to humans".
European Chemical Agency	ECHA 2016	No hazard classification for carcinogenicity is warranted	Conclusion is "based on epidemiological data as well as on data from long-term studies in rats and mice, taking a weight of evidence approach, no hazard classification for carcinogenicity is warranted for glyphosate according to the CLP criteria"
European Food Safety Authority	EFSA 2015	Unlikely to pose a carcinogenic hazard to humans	Conclusion is based on very limited evidence for an association between glyphosate-based formulations and non-Hodgkin lymphoma, overall inconclusive for a causal or clear associative relationship between glyphosate and cancer in human studies, "no evidence of carcinogenicity" in rats or mice, and "unlikely to be genotoxic".
Food and Agricultural Organization/World Health Organization Joint Meeting on Pesticide Residues	FAO and WHO 2016	Unlikely to pose a carcinogenic risk to humans from dietary exposure	Conclusions were "in view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures."
Health Canada	Health Canada 2015, 2017	Unlikely to pose a human cancer risk	In consideration of the strength and limitations of the large body of information on glyphosate, which included multiple short- and long-term (lifetime) animal toxicity studies and numerous *in vivo* and *in vitro* genotoxicity assays, as well as the large body of epidemiological information.

Table 2-13. Carcinogenicity Classification

Organization	Reference	Classification	Justification
International Agency for Research on Cancer	IARC 2017	Group 2A (*probably carcinogenic to humans)*	This classification is based on IARC's conclusions that there is "*limited evidence*" in humans, "*sufficient evidence*" in animals, and evidence that glyphosate and glyphosate-based formulations are genotoxic and capable of inducing oxidative stress.
New Zealand Environmental Protection Agency	NZ EPA 2016	Unlikely to be genotoxic or carcinogenic to humans	This conclusion is "based on a weight of evidence approach, and taking into account the quality and reliability of the available data – glyphosate is unlikely to be genotoxic or carcinogenic to humans."

glyphosate, refer to the following sources: Acquavella et al. 2016; Greim et al. 2015; McClellan 2016; Portier et al. 2016; Samsel and Seneff (2015); Tarazona et al. 2017; Williams et al. 2016.

2.20 GENOTOXICITY

The potential genotoxicity of glyphosate technical and glyphosate formulations has been extensively evaluated. The intent of this section of the Toxicological Profile for Glyphosate is to present representative results from available sources of information on glyphosate technical and glyphosate formulations. Results from selected *in vitro* and *in vivo* genotoxicity tests for glyphosate technical are presented in Tables 2-14 and 2-15, respectively. Results from selected *in vitro* and *in vivo* genotoxicity tests for glyphosate formulations are presented in Tables 2-16 and 2-17, respectively.

Table 2-14. Genotoxicity of Glyphosate Technical *In Vitro*

Species (test system)	Test substance purity	Endpoint	Result		Reference
			Activation		
			With	Without	
Salmonella typhimurium TA98, TA100, TA1535, TA1537	NS	Gene mutation	–	–	EPA 1992i
S. typhimurium TA98, TA100	NS	Gene mutation	–	–	Kubo et al. 2002
S. typhimurium TA97a, TA98, TA100, TA102	NS	Gene mutation	–	–	Chruscielska et al. 2000
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	98%	Gene mutation	–	–	Li and Long 1988
S. typhimurium TA97, TA98, TA100, TA1535	98.6%	Gene mutation	–	–	NTP 1992
Escherichia coli WP2 *hcr*	98%	Gene mutation	–	–	Li and Long 1988
Chinese hamster ovary cells	98%	Gene mutation	–	–	Li and Long 1988
Bacillus subtilis rec+, rec-	98%	*rec* assay	NT	–	Li and Long 1988
Human peripheral blood lymphocytes	>98%	Chromosomal aberrations	NT	+	Lioi et al. 1998a
Bovine peripheral blood lymphocytes	≥98%	Chromosomal aberrations	NT	+	Lioi et al. 1998b
Human peripheral blood lymphocytes	>96%	Chromosomal aberrations	NT	–	Mañas et al. 2009
Human peripheral blood lymphocytes	>98%	Sister chromatid exchange	NT	(+)	Lioi et al. 1998a

Table 2-14. Genotoxicity of Glyphosate Technical *In Vitro*

Species (test system)	Test substance purity	Endpoint	Result: Activation With	Result: Activation Without	Reference
Human peripheral blood peripheral blood	99.9%	Sister chromatid exchange	NT	+	Bolognesi et al. 1997
Bovine peripheral blood lymphocytes	≥98%	Sister chromatid exchange	NT	(+)	Lioi et al. 1998b
Human peripheral blood lymphocytes	98%	Micronuclei	+/–	–	Mladinic et al. 2009a
Human peripheral blood lymphocytes	98%	Micronuclei	+/–	–	Mladinic et al. 2009b
Human-derived buccal epithelial cells	95%	Micronuclei	NT	+	Koller et al. 2012
Chinese hamster CHO-K1 cells	NS	Micronuclei	–	+	Roustan et al. 2014
Rat hepatocytes	98%	Unscheduled DNA synthesis	NT	–	Li and Long 1988
Human fibroblast CM5757 cells	96%	DNA damage	NT	+	Alvarez-Moya et al. 2014
Human fibroblasts	98.4%	DNA damage	NT	+	Lueken et al. 2004
Human peripheral blood lymphocytes	96%	DNA damage	NT	+	Mañas et al. 2009
Human peripheral blood lymphocytes	98%	DNA damage	+	+	Mladinic et al. 2009a
Human GM38 cells	Technical grade	DNA damage	NT	+	Monroy et al. 2005
Human HT1080 (fibrosarcoma) cells	Technical grade	DNA damage	NT	+	Monroy et al. 2004, 2005
Chinese hamster ovary cells	Technical grade	DNA damage	NT	+	Monroy et al. 2004

– = negative result; + = positive result; (+) = weakly positive result; +/– = equivocal result; DNA = deoxyribonucleic acid; NS = not specified; NT = not tested

Table 2-15. Genotoxicity of Glyphosate Technical *In Vivo*

Species (test system)	Test substance purity	Endpoint	Result	Reference
		Oral exposure		
Mouse (bone marrow)	98.6%	Micronuclei	–	NTP 1992
Mouse (male germ cells)	98.7%	Dominant lethal mutation	–	EPA 1992j
		Intraperitoneal injection		
Rat (bone marrow)	98%	Chromosomal aberrations	–	Li and Long 1988

Table 2-15. Genotoxicity of Glyphosate Technical *In Vivo*

Species (test system)	Test substance purity	Endpoint	Result	Reference
Mouse (bone marrow)	99.9%	Micronuclei	+	Bolognesi et al. 1997
Mouse (bone marrow)	96%	Micronuclei	+	Mañas et al. 2009
Mouse (bone marrow)	NS[a]	Micronuclei	–	Rank et al. 1993
Mouse (liver DNA)	99.9%	DNA damage	+	Bolognesi et al. 1997
Mouse (kidney DNA)	99.9%	DNA damage	+	Bolognesi et al. 1997
Mouse (liver DNA)	99.9%	Oxidative DNA damage	+	Bolognesi et al. 1997
Mouse (kidney DNA)	99.9%	Oxidative DNA damage	–	Bolognesi et al. 1997
Mouse (liver, kidney DNA)	NS[a]	DNA adducts	–	Peluso et al. 1998

[a]Test substance: glyphosate isopropylamine salt.

– = negative result; + = positive result; DNA = deoxyribonucleic acid; NS = not specified

Table 2-16. Genotoxicity of Glyphosate Formulations *In Vitro*

Test system	Glyphosate formulation	End point	Result: Activation With	Result: Activation Without	Reference
Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Roundup® (composition NS)	Gene mutation	–	–	Moriya et al. 1983
S. typhimurium TA98	Roundup® (48% glyphosate isopropylamine salt)	Gene mutation	–	(+)[a]	Rank et al. 1993
S. typhimurium TA100	Roundup® (48% glyphosate isopropylamine salt)	Gene mutation	(+)[b]	–	Rank et al. 1993
S. typhimurium TA98, TA100	Glyphosate (Unspecified commercial formulation)	Gene mutation	–	–	Wildeman and Nazar 1982
Escherichia coli WP2 *hcr*	Roundup® (composition NS)	Gene mutation	–	–	Moriya et al. 1983
Bovine peripheral blood lymphocytes	Glyphosate (62% w/w isopropylamine salt; 38% unspecified inerts)	Chromosomal aberrations	NT	–	Holečková 2006
Bovine peripheral blood lymphocytes	Glyphosate (62% isopropylamine salt; 38% unspecified inerts)	Chromosomal aberrations	NT	–	Šiviková and Dianovský 2006

Table 2-16. Genotoxicity of Glyphosate Formulations *In Vitro*

Test system	Glyphosate formulation	End point	Result		Reference
			Activation		
			With	Without	
Human peripheral blood lymphocytes	Roundup® (not otherwise described)	Sister chromatid exchange	NT	(+)	Vigfusson and Vyse 1980
Human peripheral blood lymphocytes	Roundup® (30.4% glyphosate)	Sister chromatid exchange	NT	+	Bolognesi et al. 1997
Bovine peripheral blood lymphocytes	Glyphosate (62% isopropylamine salt; 38% unspecified inerts)	Sister chromatid exchange	+	+	Šiviková and Dianovský 2006
Human-derived buccal epithelial cells	Roundup Ultra Max® (45% glyphosate)	Micronuclei	NT	+	Koller et al. 2012
Bovine peripheral blood lymphocytes	Glyphosate (62% isopropylamine salt; 38% unspecified inerts)	Micronuclei	NT	(+)	Piešová 2004
Bovine peripheral blood lymphocytes	Glyphosate (62% isopropylamine salt; 38% unspecified inerts)	Micronuclei	NT	(+)	Piešová 2005
Human liver HepG2 cells	Grands Travaux® (40% glyphosate)	DNA damage	NT	(+)	Gasnier et al. 2009
E. coli PQ37	Roundup BIO® (NS)	DNA damage	NT	+	Raipulis et al. 2009

[a]Weakly positive at 360 µg/plate in one test (4-fold increase in revertants/plate) but not in another test; cytotoxicity at concentrations ≥360 µg/plate.
[b]Weakly positive at 720 µg/plate (3.3-fold increase in revertants/plate); cytotoxicity at concentrations ≥360 µg/plate.

– = negative result; + = positive result; (+) = weakly positive result; NS = not specified; NT = not tested

Table 2-17. Genotoxicity of Glyphosate Formulations *In Vivo*

Species (test system)	Test substance (purity)	End point	Result	Reference
Drosophila (sex-linked recessive lethal mutation assay)[a]	Roundup® (glyphosate isopropylamine salt; purity NS)	Gene mutation	+	Kale et al. 1995
		Oral		
Drosophila (somatic mutation assay)	Roundup® (NS)	Gene mutation	+	Ramos-Morales et al. 2008
Mouse (bone marrow)	Roundup® (9.8% active ingredient)	Chromosomal aberrations	–	Dimitrov et al. 2006

Table 2-17. Genotoxicity of Glyphosate Formulations *In Vivo*

Species (test system)	Test substance (purity)	End point	Result	Reference
		Intraperitoneal injection		
Mouse (bone marrow)	Roundup® (>41% glyphosate isopropylamine salt)	Chromosomal aberrations	+	Prasad et al. 2009
Mouse (bone marrow)	Roundup® (48% glyphosate isopropylamine salt)	Micronuclei	–	Rank et al. 1993
Mouse (bone marrow)	Roundup® (30.4% glyphosate)	Micronuclei	+	Bolognesi et al. 1997
Mouse (bone marrow)	Roundup® (9.8% glyphosate)	Micronuclei	–	Dimitrov et al. 2006
Mouse (bone marrow)	Roundup® (>41% glyphosate isopropylamine salt)	Micronuclei	+	Prasad et al. 2009
Mouse (bone marrow)	Roundup® (48% glyphosate isopropylammonium salt; 12% inerts including POEA)	Micronuclei	–	Grisolia 2002
Mouse (bone marrow)	Roundup® (NS)	Micronuclei	+	Rodrigues et al. 2011
Mouse (liver DNA)	Roundup® (30.4% glyphosate)	DNA damage	+	Bolognesi et al. 1997
Mouse (kidney DNA)	Roundup® (30.4% glyphosate)	DNA damage	+	Bolognesi et al. 1997
Mouse (liver DNA	Roundup® (30.4% glyphosate)	Oxidative DNA damage	–	Bolognesi et al. 1997
Mouse (kidney DNA)	Roundup® (30.4% glyphosate)	Oxidative DNA damage	+	Bolognesi et al. 1997
Mouse (liver, kidney DNA)	Roundup® (30.4% glyphosate isopropylammonium salt)	DNA adducts	+	Peluso et al. 1998

[a]*Drosophila* larvae were exposed to test substance in growing medium.

+ = positive result; – = negative result; DNA = deoxyribonucleic acid; NS = not specified

Glyphosate Technical. Glyphosate did not induce gene mutations either with or without exogenous metabolic activation in numerous bacterial assays, or in assays using mammalian cells (Chruscielska et al. 2000; EPA 1992i, Kubo et al. 2002; Li and Long 1988; NTP 1992). Lioi et al. (1998a, 1998b) reported concentration-related significant increases in chromosomal aberrations in human and bovine peripheral blood lymphocytes exposed to glyphosate, although concomitant decreases in mitotic index were indicative of some degree of cytotoxicity at least at the highest glyphosate concentrations. Mañas et al.

(2009) found no evidence of glyphosate-induced chromosomal aberrations in human peripheral blood lymphocytes. Glyphosate was positive for induction of sister chromatid exchange in one assay using human peripheral blood lymphocytes (Bolognesi et al. 1997); weakly positive responses were obtained in other assays using human lymphocytes (Lioi et al. 1998a) and bovine lymphocytes (Lioi et al. 1998b). There was some evidence of cytotoxicity in the assays of Lioi et al. (1998a, 1998b). Glyphosate did not induce micronuclei in human peripheral blood lymphocytes exposed to glyphosate in the absence of exogenous metabolic activation; an equivocal result was obtained in the presence of exogenous metabolic activation (Mladinic et al. 2009a, 2009b). The result was considered equivocal due to significant apoptosis at concentrations resulting in significantly increased micronuclei frequency. Koller et al. (2012) reported significantly increased frequency of micronuclei in an assay using human-derived buccal epithelial cells exposed to glyphosate. Roustan et al. (2014) reported significantly increased micronuclei frequency in Chinese hamster ovary K1 cells exposed to glyphosate without (but not with) exogenous metabolic activation. Negative results were obtained in an assay that evaluated the potential for glyphosate to induce unscheduled DNA synthesis in rat hepatocytes (Li and Long 1988). Mañas et al. (2009) and Lueken et al. (2004) reported positive results for DNA damage in glyphosate-exposed human fibroblasts. Exposure concentration-related significantly increased frequency of DNA damage was observed in another assay of glyphosate-exposed human peripheral blood lymphocytes, although significant apoptosis observed at all concentrations resulting in increased DNA damage (Mladinic et al. 2009a). Alvarez-Moya et al. (2014) reported DNA damage in human fibroblast CM5757 cells exposed to glyphosate technical. Exposure-related DNA damage was observed in assays of human GM38 cells (Monroy et al. 2005), human HT1080 (fibrosarcoma) cells (Monroy et al. 2004, 2005), and Chinese hamster ovary cells (Monroy et al. 2004) exposed to glyphosate technical.

The genotoxicity of glyphosate technical has been evaluated in a number of *in vivo* tests; results are mixed across a variety of cell types. Glyphosate did not induce dominant lethal mutations following oral dosing of male CD-1 mice once by gavage at up to 2,000 mg/kg (EPA 1992j). Glyphosate did not increase the frequency of micronuclei in bone marrow cells from B6C3F1 mice administered glyphosate in the diet for 13 weeks at concentrations resulting in estimated doses as high as 10,780-11,977 mg/kg/day (NTP 1992). Glyphosate did not increase the frequency of micronuclei in bone marrow cells from C3H mice administered glyphosate technical via single intraperitoneal injection (Chruscielska et al. 2000) or NMRI-bom mice administered glyphosate (as isopropylammonium salt) via two intraperitoneal injections 24 hours apart (Rank et al. 1993). Glyphosate did not induce chromosomal aberrations in bone marrow cells from rats administered glyphosate via intraperitoneal injection at 1,000 mg/kg (Li and Long 1988). Kier and Kirkland (2013) summarized results from 10 industry studies

that evaluated frequency of micronuclei in bone marrow cells from mice or rats administered glyphosate orally or via intraperitoneal injection; results were consistently negative for glyphosate-induced micronuclei, although an inconclusive result was determined for one study. However, other investigators reported positive results for micronuclei induction in bone marrow cells from mice administered glyphosate via intraperitoneal injection by single 300 mg/kg dose (Bolognesi et al. 1997) or two 200 mg/kg doses 24 hours apart (Mañas et al. 2009). Bolognesi et al. (1997) reported significantly increased frequency of DNA damage (single strand breaks) in liver and kidney and significantly increased frequency of oxidative DNA damage in liver (but not kidney) from mice administered glyphosate via single intraperitoneal injection at 300 mg/kg. Peluso et al. (1998) found no evidence of the formation of DNA adducts in liver or kidney from mice following intraperitoneal injection of glyphosate (as isopropylammonium salt) at up to 270 mg/kg. It should be noted that intraperitoneal injection studies typically employed lethal dose levels; a positive result at such high dose levels does not necessarily indicate potential for genotoxicity at doses relevant to human exposure.

DNA damage in human fibroblast cells and peripheral blood lymphocytes were the most frequently reported clearly positive results from available *in vitro* assays that employed glyphosate technical. From available *in vivo* assays that employed glyphosate technical, DNA damage in mouse kidney and liver was the most frequent positive result. Summaries should be interpreted with caution because the genotoxicity of glyphosate technical was assessed based on a limited number of primary results available to ATSDR.

Glyphosate Formulations. Glyphosate formulations (active ingredient typically ranging from approximately 30 to 62% of the formulation) were not mutagenic to bacterial test systems in available published studies (Chruscielska et al. 2000; Moriya et al. 1983; Wildeman and Nazar 1982), numerous unpublished industry studies summarized by Kier and Kirkland (2013), or several other studies summarized by Williams et al. (2000). Weakly positive results were obtained for *Salmonella typhimurium* strain TA98 in the absence (but not presence) of exogenous metabolic activation and strain TA100 in the presence (but not absence) of exogenous metabolic activation (Rank et al. 1993); however, the positive responses were observed at concentrations exhibiting cytotoxicity and in only one of two tests in strain TA98. Roundup® did not induce chromosomal aberrations in bovine peripheral blood lymphocytes in two assays that employed 24-hour exposures (Holečková 2006; Šiviková and Dianovský 2006); however, a significant increase in sister chromatid exchange was noted both with and without exogenous metabolic activation (Šiviková and Dianovský 2006). A slight, (statistically significant) 1.1–1.3-fold increase in frequency of sister chromatid exchange was observed in human peripheral blood lymphocytes exposed to Roundup® (Vigfusson and Vyse 1980). Bolognesi et al. (1997) reported

significantly increased sister chromatid exchange (1.3–1.5-fold greater than that of controls) in human peripheral blood lymphocytes exposed to Roundup® for 72 hours at concentrations of 0.1 and 0.33 mg/mL. The magnitude of this effect was comparable to that obtained using analytical-grade glyphosate at 10 times the concentration of the Roundup® formulation, indicating that other substances in the Roundup® formulation may have been at least partly responsible for the effect. In two assays, Roundup® induced micronuclei in cultured bovine peripheral blood lymphocytes at noncytotoxic concentrations (Piešová 2004, 2005). Koller et al. (2012) reported significantly increased numbers of micronuclei in human-derived buccal epithelial cells exposed to Roundup Ultra Max® for 20 minutes, including concentrations that were noncytotoxic; this effect was more pronounced than that resulting from similar treatment using analytical grade glyphosate. A weakly positive result for DNA damage was reported for human liver HepG2 cells exposed to Roundup Grands Travaux® (Gasnier et al. 2009). Exposure to non-specified concentrations of glyphosate resulted in treatment-related DNA damage in *Escherichia coli* PQ37 cells (Raipulis et al. 2009).

Several studies were designed to evaluate the genotoxicity of selected glyphosate formulations *in vivo*; similar to findings from *in vivo* studies using glyphosate technical, mixed results were obtained from *in vivo* exposure to glyphosate-containing products. Roundup® induced mutations in *Drosophila* in a sex-linked recessive lethal mutation assay (Kale et al. 1995) and a somatic mutation assay (Ramos-Morales et al. 2008). Roundup® did not induce chromosomal aberrations or micronuclei in mice administered the test chemical orally at a 1,080 mg/kg dose, reported by the study authors as one-half the LD_{50} (Dimitrov et al. 2006). The potential for Roundup® to induce chromosomal aberrations and/or micronuclei in bone marrow cells has been assessed in several studies in which the test chemical was administered to mice via intraperitoneal injection. Although intraperitoneal administration of Roundup® at 25 and 50 mg/kg resulted in significantly increased frequencies of chromosomal aberrations and micronuclei, both doses appeared to be cytotoxic, as indicated by time- and dose-related significant decreases in mitotic indices (Prasad et al. 2009). Rodrigues et al. (2011) reported significantly increased micronucleus frequency at intraperitoneal doses of 0.754 and 1.28 mg/kg for Roundup®; the response was reported to be as pronounced as that of a positive control substance (250 mg cyclophosphamide/kg). Roundup® induced micronuclei in bone marrow from mice administered the chemical via intraperitoneal injection at 300 mg/kg (expressed as glyphosate) (Bolognesi et al. 1997). Negative results were reported in two other studies that evaluated micronucleus induction in bone marrow cells from mice treated by intraperitoneal injection of Roundup® (Grisolia 2002; Rank et al. 1993). In the study of Grisolia (2002), polyoxyethylene amine surfactant accounted for 12% of the formulation. Negative results were also reported for micronucleus induction in bone marrow cells from mice treated by intraperitoneal injection

of a commercial formulation identified only as Perzocyd 10 SL (Chruscielska et al. 2000). Roundup® induced single-strand breaks in DNA from liver and kidney of mice administered the chemical via intraperitoneal injection at 300 mg/kg (expressed as glyphosate) and oxidative DNA damage in kidney (but not liver) cells (Bolognesi et al. 1997). However, Heydens et al. (2008) repeated the study design of Bolognesi et al. (1997) and found a 300 mg/kg intraperitoneally-injected dose to be highly toxic to liver and kidney. It was suggested that the genotoxic effects observed by Bolognesi et al. (1997) might have been secondary effects mediated by local toxicity. Peluso et al. (1998) reported the formation of DNA adducts in liver and kidney from mice following intraperitoneal injection of Roundup® at doses in the range of 122–182 mg active ingredient/kg. The DNA adduct formation was considered likely related to other components of the Roundup® formulation because DNA adduct formation was not observed in mice similarly treated with analytical-grade glyphosate at 270 mg/kg.

Exposure to glyphosate-containing products and evidence of genetic damage was reported in limited human studies. Paz-y-Miño et al. (2007) evaluated prevalence of DNA strand breaks in blood samples from 24 residents of an area in northern Ecuador at 2 weeks to 2 months following aerial applications of Roundup-Ultra®; the study included 21 unexposed control individuals. The exposed individuals exhibited a higher degree of DNA damage (comet length 35.5±6.4 μm) than the unexposed controls (comet length 25.94±0.6 μm). There was no evidence of exposure-related chromosomal damage among 92 individuals from 10 communities near the northern Ecuador border evaluated at 2 years following the last aerial applications of glyphosate-containing herbicides (Paz-y-Miño et al. 2011). Bolognesi et al. (2009) reported increases in micronuclei in peripheral blood lymphocytes from nearby residents following aerial spraying of glyphosate-based formulation with adjuvant to coca and poppy crops, or without adjuvant on sugar-cane plantations. These residents were evaluated both prior to and following aerial spraying.

DNA damage in human cells was the most frequently reported clearly positive results from available *in vitro* assays that employed glyphosate formulations. However, comparison of results across available studies was precluded due to lack of information regarding the composition of the various formulations tested. From available *in vivo* assays that employed glyphosate formulations, DNA damage in mouse kidney and liver was the most frequent positive result. Summaries should be interpreted with caution because the genotoxicity of glyphosate technical was assessed based on a limited number of primary results available to ATSDR.

Additional unpublished genotoxicity assays were submitted to EPA and/or the European Commission (EC) during re-registration of products containing glyphosate. Many agencies, organizations, and/or expert panels have reviewed available genotoxicity data and concluded that the data do not support a genotoxicity role for glyphosate, at least at concentrations relevant to human exposure (e.g., APVMA 2017; Brusick et al. 2016; EFSA 2015; EPA 2017c; FAO and WHO 2016; Health Canada 2017; Kier and Kirkland 2013; NZ EPA 2016; Williams et al. 2016). In contrast, IARC (2017) concluded that there is strong evidence for the genotoxicity of glyphosate. For more detailed information regarding genotoxicity evaluations and conclusions of these agencies, organizations, and/or expert panels, consult corresponding references.

2.21 MECHANISMS OF ACTION

Mechanism of Action in Plants. Glyphosate-based herbicides act on the shikimate pathway in plants by blocking the activity of the enzyme, 5-enolpyruvylshikimate-3-phosphate synthetase (EPSPS), and thereby inhibiting the biosynthesis of essential aromatic amino acids in plants (see Funke et al. 2006; Martinez et al. 2018; Pollegioni et al. 2011 for more specific information regarding mechanisms of action). The action of glyphosate on the shikimate pathway is not of direct human concern because this pathway does not exist in mammals.

Some crop plants have been genetically modified to resist the action of glyphosate by the addition of a glyphosate-insensitive form of EPSPS (CP4 EPSPS) obtained from *Agrobacterium* sp. strain CP4 (Funke et al. 2006). Some transgenic plants have been genetically altered to express N-acetyltransferase proteins (e.g., glyphosate acetyltransferase [GAT4601] from *Bacillus licheniformis*), which acetylate glyphosate to a non-phytotoxic metabolite (N-acetylglyphosate) (Pioneer 2006).

Proposed Mechanisms of Action with Human Relevance. Although glyphosate is generally considered to be of relatively low toxicity to mammals, the following mechanisms of action have been proposed:

Hepatotoxicity. Ford et al. (2017) administered glyphosate to male C57BL/6 mice by intraperitoneal injection at 200 mg/kg/day for 7 days, after which livers were evaluated for levels of glyphosate, AMPA, and glyoxylate (a reactive substance produced endogenously). Glyphosate treatment at this high dose level resulted in measurable levels of AMPA, indicating some degree of glyphosate metabolism. Glyphosate treatment also resulted in an approximately 2-fold increase in glyoxylate. Because glyoxylate is formed endogenously, the increase in glyoxylate level in the liver may be a result of glyphosate acting

on mechanisms responsible for endogenous production of glyoxylate. The study authors demonstrated that glyoxylate inhibited liver fatty acid oxidation enzymes in mice and that glyphosate treatment increased triglycerides and cholesteryl esters, which was considered a likely result of the diversion of fatty acids toward lipid pathways other than oxidation.

Renal toxicity. Mohamed et al. (2016) observed increases in serum and urinary cystatin C and urinary interleukin-18, cytochrome C, and neutrophil gelatinase-associated protein (NGAL) in patients presenting with poisoning from glyphosate-based formulations. The study authors noted that the increases in cystatin C and interleukin-18 suggest that glyphosate-based formulations might induce apoptosis and mitochondrial toxicity.

Dedeke et al. (2018) administered glyphosate alone or a glyphosate-based formulation to rats by daily gavage for 12 weeks at dose levels of 3.6, 50.4, or 248.8 mg glyphosate/kg/day. The rats administered the glyphosate-based formulation exhibited significantly altered markers of kidney changes (serum urea and creatinine, plasma cystatin-C, NGAL), oxidative stress, and activities of selected membrane-bound enzymes compared to the rats treated with glyphosate alone. Those rats administered glyphosate-based formulation were the only ones to exhibit severe histopathologic kidney lesions. The study authors suggested that these results did not support a nephrotoxic role for glyphosate alone.

Neurotoxicity. Cattani et al. (2014) added 1% Roundup® (0.38% glyphosate) to the drinking water of rat dams from gestation day 5 through lactation day 15. Hippocampal slices from 15-day-old pups were exposed to Roundup® (0.00005–0.1%) for 30 minutes. The study authors reported that Roundup® treatment resulted in increased Ca^{2+} influx via activation of NMDA receptors and voltage-dependent Ca^{2+} channels, activation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and extracellular signal-regulated kinase (ERK), increased glutamate release into the synaptic cleft, decreased glutathione content, increased lipoperoxidation, decreased glutamate uptake and metabolism, and induced Ca^{2+} uptake and methyl-amino-isobutyric acid accumulation. The study authors suggested that exposure to Roundup® might lead to excessive extracellular glutamate levels and resulting glutamate excitotoxicity and oxidative stress in rat hippocampus.

Reproductive/endocrine effects. Perego et al. (2017) reported results from an *in vitro* study designed to evaluate the effects of glyphosate treatment (up to 5 µg/mL) on bovine granulosa cells and theca cells. Granulosa cell proliferation and estradiol production were impaired, but no effects were observed on

theca cell proliferation or steroidogenesis. The results suggest that glyphosate may affect the reproductive system in cattle via direct action on ovarian function.

Romano et al. (2010) reported decreased serum testosterone in young male rats gavaged with Roundup Transorb®. Romano et al. (2012) implicated disruption of gonadotropin expression as a mechanism of action.

Carcinogenicity. As stated in Section 2.20 (Genotoxicity), IARC (2017) concluded that there is strong evidence for the genotoxicity of glyphosate, although other agencies, organizations, and/or expert panels have concluded that the data do not support a genotoxicity role for glyphosate (e.g., APVMA 2017; Brusick et al. 2016; EFSA 2015; EPA 2017c; FAO and WHO 2016; Health Canada 2017; Kier and Kirkland 2013; NZ EPA 2016; Williams et al. 2016). IARC (2017) also concluded that there is strong evidence for glyphosate-induced oxidative stress based on results from studies of animal models *in vivo* and human cells *in vitro*.

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Toxicokinetic data for glyphosate are summarized below.

- Glyphosate is readily absorbed from the gastrointestinal tract; very little glyphosate is absorbed through the skin; it is assumed that glyphosate is readily absorbed from the respiratory tract.
- Absorbed glyphosate is readily distributed via the blood, but does not accumulate in any particular organ or tissue.
- Glyphosate does not undergo significant metabolism in mammals; <1% is metabolized to AMPA.
- Approximately two-thirds of an oral dose of glyphosate is excreted in the feces as unabsorbed parent compound. Most absorbed glyphosate is rapidly excreted in the urine as parent compound.

3.1.1 Absorption

3.1.1.1 Inhalation Exposure

Limited information is available regarding the toxicokinetics of inhaled glyphosate. Observations of increased urinary glyphosate levels among 48 farmer-applicators following application of glyphosate-containing products is evidence that inhaled glyphosate can be absorbed (Acquavella et al. 2004). However, dermal absorption was likely involved in some cases because mean urinary glyphosate was higher among those farmers (14/48) who did not use rubber gloves. Detectable levels of urinary glyphosate were also measured in children of the farmers who were present during mixing, loading, or application of the herbicide; exposures among the children may have involved inhalation and/or dermal routes. No information was located regarding the toxicokinetics of inhaled glyphosate in among laboratory animals.

3.1.1.2 Oral Exposure

Information regarding the toxicokinetics of ingested glyphosate in humans is limited. The detection of glyphosate in serum and/or urine samples from individuals who had intentionally or unintentionally ingested glyphosate-containing products is confirmation of absorption from the gastrointestinal tract (e.g., Hiraiwa et al. 1990; Hori et al. 2003; Sribanditmongikol et al. 2012; Zouaoui et al. 2013). Numerous reports of systemic effects following intentional or unintentional ingestion of glyphosate-

containing products serve as additional evidence that ingested glyphosate is absorbed (e.g., Chang and Chang 2009; Chen et al. 2009; Hsiao et al. 2008; Kim et al. 2014; Lee et al. 2000; Menkes et al. 1991; Moon and Chun 2010; Roberts et al. 2010; Sato et al. 2011; Sawada et al. 1988; Sørenson and Gregersen 1999; Stella and Ryan 2004; Talbot et al. 1991; Tominack et al. 1991).

Several groups of investigators have evaluated the absorption of glyphosate following oral exposure of laboratory animals, particularly rats. In one study (NTP 1992), male F344/N rats were administered a single gavage dose of ^{14}C-glyphosate (purity 99%) in distilled water at 5.6 or 56 mg/kg. Other rats were administered a single dose of glyphosate at 5.6 mg/kg via intravenous injection, intraperitoneal injection, or oral (gavage) to compare 24-hour urinary and fecal elimination by these administration routes. Results from comparative studies of oral, intravenous, and intraperitoneal administration of glyphosate indicated that urinary radioactivity represented the amount of glyphosate absorbed and fecal radioactivity represented the amount of unabsorbed glyphosate following oral exposure. Although quantitative data were not included in the study report, the study authors estimated that 30% of the 5.6 mg/kg dose of ^{14}C-glyphosate was absorbed and that a slightly higher percentage (34%) of the 56 mg/kg dose was absorbed. In another study, male Sprague-Dawley rats received a single gavage dose of ^{12}C- and ^{14}C-glyphosate at 10 mg/kg (Brewster et al. 1991). Based on urinary radioactivity, it was estimated that 35–40% of the oral dose had been absorbed from the gastrointestinal tract. Anadón et al. (2009) reported an absorption half-life of 2.29 hours following administration of an oral dose of 400 mg glyphosate/kg to rats; an estimated peak plasma glyphosate of 4.62 µg/mL was reached at 5.16 hours postdosing. Results from a number of unpublished industry studies cited in EPA (1993), FAO and WHO (2016), IPCS (1994), and/or Williams et al. (2000), but not available to ATSDR, demonstrate that single or repeated oral dosing of glyphosate to rats at doses in the range of 10–1,000 mg/kg/day result in urinary excretion of 7–36% of the administered dose during ≤7 days of posttreatment, which presumably represents the proportion of absorbed glyphosate.

3.1.1.3 Dermal Exposure

Limited human data are available regarding the toxicokinetics of glyphosate following dermal exposure. Increased urinary glyphosate levels among 48 farmer-applicators following application of glyphosate-containing products is evidence that glyphosate can be absorbed (Acquavella et al. 2004). Dermal absorption was likely involved in some cases because mean urinary glyphosate was higher among those farmers (14/48) who did not use rubber gloves.

In vitro studies using human skin samples indicate that dermal penetration of glyphosate is very low. Wester et al. (1996) applied 300 µL of a 1% aqueous dilution of analytical-grade ^{14}C-labeled glyphosate to human cadaver skin (0.8 cm^2 of available skin area). The study authors reported a permeability constant of 4.59x10^{-4} cm/hour, with a lag time of 10.48 hours, which resulted in a calculated flux of 4.12 µg glyphosate/hour. Wester et al. (1991) used a ^{14}C-labeled Roundup® formulation to evaluate dermal absorption of glyphosate through human skin (*in vitro*) and abdominal skin of Rhesus monkeys (*in vivo*). Undiluted application to human skin samples at doses ranging from 15.4 to 154 µg/cm^2 resulted in 0–0.4% dermal absorption over 8 hours postapplication; dermal absorption of glyphosate from aqueous dilutions of test substance (1:20 or 1:32 test substance:water, v/v) during 16 hours postapplication was ≤2.2%. Twelve-hour *in vivo* application of the test substance diluted 1:29 with water at concentrations of 25 or 270 µg/cm^2 resulted in 7-day recovery of 0.8 and 2.2% of the applied dose, respectively, in the urine and 3.6 and 0.7%, respectively, in the feces. These results indicate that approximately 3–4% of the applied dose had been absorbed.

3.1.2 Distribution

3.1.2.1 Inhalation Exposure

No human or animal data were located regarding distribution of glyphosate following absorption via the inhalation exposure route.

3.1.2.2 Oral Exposure

Limited human data were located regarding distribution of glyphosate following absorption via the oral exposure route. Menkes et al. (1991) reported measurable glyphosate in kidney, liver, blood, and brain in postmortem examination of an individual who had ingested 200–250 mL of Roundup®.

Following oral administration, absorbed glyphosate is readily distributed and rapidly eliminated without significant accumulation in any particular tissue. In male F344/N rats administered single gavage dose of ^{14}C-glyphosate (purity 99%) in distilled water at 5.6 or 56 mg/kg, peak blood radioactivity occurred at 1 and 2 hours postdosing, respectively, mean peak blood concentration was 30-fold higher in the high-dose group (NTP 1992). Among rats gavaged at 5.6 mg radiolabeled glyphosate/kg and evaluated for tissue distribution, total tissue radioactivity amounted to approximately 12, 11.7, 5.5, 0.9, and 0.1% of the administered dose at 3, 6, 12, 24, and 96 hours postdosing, respectively. The highest radioactivity level was found in the small intestine, reaching a peak level of approximately 10% of the administered dose at

6 hours postdosing; radioactivity in the large intestine peaked at approximately 1.2% at 3 hours postdosing. Liver, kidney, skin, and blood each accounted for <1% of the administered dose at each time point. By 24 hours postdosing, <1% of the administered dose remained in all tissues combined. Brewster et al. (1991) administered ^{12}C- and ^{14}C-glyphosate by single gavage dose at 10 mg/kg to male Sprague-Dawley rats and found approximately 34% of the administered dose in the small intestine (not associated with intestinal content) at 2 hours postdosing, decreasing to 0.05% of the administered dose by 96 hours postdosing. Radioactivity levels in most other tissues (blood, colon, kidney, liver, stomach, abdominal fat, testicular fat) peaked at 2–6 hours postdosing; each of these tissues accounted for ≤1.3% of the administered dose at peak and ≤0.06% by 96 hours postdosing. Radioactivity in bone peaked at 6 hours postdosing (4.7% of the administered dose) and remained at 1.7% at 96 hours postdosing. The tissue to blood ratio for bone increased with time suggesting a slower elimination from bone compared to blood. Anadón et al. (2009) reported an absorption half-life of 2.29 hours following administration of an oral dose of 400 mg glyphosate/kg to rats; an estimated peak plasma glyphosate of 4.62 µg/mL was reached at 5.16 hours postdosing.

3.1.2.3 Dermal Exposure

No human data were located regarding distribution following dermal exposure to glyphosate.

Limited animal data are available. The observation of radioactivity in urine and feces collected from rhesus monkeys following dermal application of a ^{14}C-labeled Roundup® formulation is demonstration of systemic distribution following dermal absorption (Wester et al. 1991). However, at sacrifice 7 days posttreatment, no radioactivity was detected in spleen, ovaries, kidney, brain, abdominal fat, bone marrow, upper spinal column, or central nervous fluid.

3.1.2.4 Other Routes of Exposure

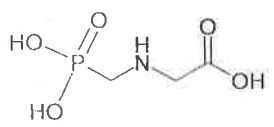
Limited data are available regarding the distribution of parenterally-administered glyphosate. Male and female Sprague-Dawley rats were administered ^{14}C-glyphosate via intraperitoneal injection at 1,150 mg/kg (EPA 1992h). Radioactivity measured in bone marrow samples taken 30 minutes postinjection amounted to approximately 0.0044 and 0.0075% of the administered activity for the males and females, respectively. Anadón et al. (2009) administered glyphosate (95% purity) to male Wistar rats via intravenous injection at 100 mg/kg. Plasma levels of glyphosate and its metabolite, AMPA, were measured using high-performance liquid chromatography (HPLC). Reported fast plasma distribution

(half-life of 0.345 hours) and high volume of distribution at steady state (2.99 L/kg) were interpreted to indicate that glyphosate was extensively distributed to extravascular tissues.

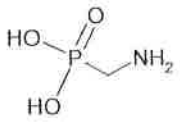
3.1.3 Metabolism

Glyphosate does not undergo significant metabolism in mammals. Available data are limited to the oral exposure route and indicate that ingested glyphosate is eliminated mostly as parent compound; only a small amount may be metabolized to AMPA. Figure 3-1 depicts the chemical structures of glyphosate and AMPA. In one human case of intentional ingestion of an herbicide in a suicide attempt, glyphosate and its metabolite, AMPA, were detected in serum and urine (Hori et al. 2003). At 16 hours postingestion, serum levels of glyphosate and AMPA were 4.4 and 0.03 µg/mL, respectively (147:1, glyphosate:AMPA). Total urinary excretion of glyphosate and its metabolite during 4 days postingestion was 3.7 g and 25 mg, respectively (148:1, glyphosate:AMPA).

Figure 3-1. Chemical Structures of Glyphosate and Aminomethylphosphonic Acid (AMPA)



Glyphosate



Aminomethylphosphonic acid (AMPA)

Results from available animal studies also indicate that very little ingested glyphosate is metabolized. Anadón et al. (2009) administered glyphosate (95% purity) to male Wistar rats by gavage at 400 mg glyphosate/kg. Plasma glyphosate peaked at 5.16 hours postdosing and measured 4.62 µg/mL; plasma AMPA peaked at 2.42 hours postdosing and measured 0.416 µg/mL. Based on the ratios between the area under the curve (AUC) for AMPA and the AUC for glyphosate, it was estimated that the metabolite represented 6.49% of the parent compound plasma concentration. In an unpublished study summarized by EPA (1993) and Williams et al. (2000), following oral administration of radiolabeled glyphosate (>99% purity) to Sprague-Dawley rats at 10 mg/kg, the glyphosate metabolite (AMPA) was detected in the urine (0.2- 0.3% of the administered dose) and feces (0.2–0.4% of the administered dose). The formation of AMPA was thought to have occurred in the gastrointestinal tract (possibly by microflora) because AMPA was not detected in other rats administered glyphosate via intravenous injection. Following a single gavage dose of administered radiolabeled glyphosate (>99% purity) to Sprague-

Dawley rats, expired air accounted for <0.27% of the administered radioactivity at 24 hours postdosing, indicating that glyphosate metabolism had occurred to a slight extent (EPA 1993).

Ford et al. (2017) administered glyphosate to male C57BL/6 mice by intraperitoneal injection at 200 mg/kg/day for 7 days. Glyphosate treatment at this high dose level resulted in measurable levels of AMPA (approximately 4% of the dose of glyphosate) and an approximately 2-fold increase in hepatic glyoxylate (a reactive substance produced endogenously). Because glyoxylate is formed endogenously, the increase in glyoxylate level in the liver may be a result of glyphosate acting on mechanisms responsible for endogenous production of glyoxylate.

3.1.4 Excretion

3.1.4.1 Inhalation Exposure

Limited information is available regarding elimination and excretion of glyphosate in humans following inhalation exposure. In one study, urinary glyphosate levels were evaluated in 48 farmer-applicators prior to application of glyphosate-containing products, immediately following application, and for 3 days thereafter (Acquavella et al. 2004). Urinary glyphosate was detectable in 15% (7/47) of the farmers prior to application, in 60% (29/48) of the farmers immediately following application, and in only 27% (13/48) of the farmers on postapplication day 3. No information was located regarding elimination or excretion following inhalation exposure of laboratory animals to glyphosate.

3.1.4.2 Oral Exposure

Roberts et al. (2010) estimated a half-life of 3–4 hours for elimination of glyphosate from the blood of patients who had intentionally ingested large amounts of glyphosate-containing herbicide products. In other cases of poisoning victims, plasma glyphosate levels dropped rapidly (within 2–3 days) following the onset of observation (e.g., Talbot et al. 1991). Glyphosate has been detected in feces and urine of individuals who intentionally or accidentally ingested relatively large amounts of glyphosate.

Results from animal studies identify the feces and urine as major routes of elimination following oral exposure to glyphosate. For example, among male and female Sprague-Dawley rats administered ^{14}C-glyphosate (99% purity) via single gavage dose at 10 mg/kg, during 7 days posttreatment, radioactivity recovered in the feces averaged 62.4 and 69.4% of the administered dose (males and females, respectively); another 28.6 and 22.5% of the administered dose (males and females, respectively)

was recovered in the urine (IPCS 1994). Thus, feces and urine accounted for approximately 88–91% of the administered dose. HPLC analysis revealed that parent compound accounted for 98.5–99.3% of the radioactivity in feces and urine. There were no significant differences in fecal and urinary excretion among rats dosed with unlabeled glyphosate for 14 days followed by a single oral dose of radiolabeled glyphosate. Following single gavage dosing of ^{14}C-glyphosate (>96% purity) to male and female Sprague-Dawley rats at 30 mg/kg, the feces accounted for 57–59% of the administered radioactivity and the urine accounted for 27–29% during the first 36 hours posttreatment; indicating that fecal and urinary excretion occur relatively rapidly following oral exposure to glyphosate (IPCS 1994). In male F344/N rats administered single gavage dose of ^{14}C-glyphosate (purity 99%) in distilled water at 5.6 or 56 mg/kg, 72-hour collection of feces and urine resulted in the recovery of 91–92% of the administered radioactivity; 74 and 19%, respectively, at the low dose and 58 and 34%, respectively, at the high dose (NTP 1992). In one study (NTP 1992), male F344/N rats were administered a single dose of glyphosate at 5.6 mg/kg via intravenous injection, intraperitoneal injection, or oral (gavage) to compare 24-hour urinary and fecal elimination by these administration routes. Results from comparative studies of oral, intravenous, and intraperitoneal administration of glyphosate indicated that urinary radioactivity represented the amount of glyphosate absorbed and fecal radioactivity represented the amount of unabsorbed glyphosate following oral exposure. Although quantitative data were not included in the study report, the study authors estimated that 30–34% of the oral doses of ^{14}C-glyphosate was absorbed and excreted in the urine. Therefore, approximately 66–70% was unabsorbed and eliminated in the feces.

Very little ingested glyphosate is eliminated via routes other than feces and urine. Among Sprague-Dawley rats administered radiolabeled glyphosate (>99% purity) by single gavage dose, <0.27% of the administered radioactivity was recovered in expired air at 24 hours postdosing (EPA 1993).

3.1.4.3 Dermal Exposure

No information was located regarding elimination or excretion following known dermal exposure to glyphosate in humans. However, in a study that evaluated urinary glyphosate levels in 48 farmer-applicators involved in application of glyphosate-containing products, mean urinary glyphosate was higher among those farmers (14/48) who did not use rubber gloves, indicating that some glyphosate had been absorbed through the skin (Acquavella et al. 2004). Limited information is available for laboratory animals. Wester et al. (1991) applied a ^{14}C-labeled Roundup® formulation to the abdominal skin of Rhesus monkeys (*in vivo*) to evaluate dermal absorption of glyphosate. Twelve-hour application of the

test substance at concentrations of 25 or 270 μg/cm^2 resulted in 7-day recovery of 0.8 and 2.2% of the applied dose, respectively, in the urine and 3.6 and 0.7%, respectively, in the feces.

3.1.4.4 Other Routes of Exposure

Male and female Sprague-Dawley rats were administered ^{14}C-glyphosate via intraperitoneal injection at 1,150 mg/kg (EPA 1993). Assuming first-order kinetics, the half-life of elimination from the bone marrow was estimated at 7.6 and 4.2 hours for the males and females, respectively. A half-life for elimination of radioactivity from plasma was approximately 1 hour for both sexes. These results indicate that glyphosate reaching the blood was rapidly eliminated and that the small fraction reaching bone marrow was rapidly eliminated. Anadón et al. (2009) reported a half-time of 9.99 hours for elimination of glyphosate from the blood of male Wistar rats administered glyphosate (95% purity) via intravenous injection at 100 mg/kg.

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

PBPK models for glyphosate were not located.

3.1.6 Animal-to-Human Extrapolations

No information was located to suggest significant differences between animals and humans regarding the toxicokinetics of glyphosate.

3.2 CHILDREN AND OTHER POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Children may be more or less susceptible than adults to health effects from exposure to hazardous substances and the relationship may change with developmental age.

This section also discusses unusually susceptible populations. A susceptible population may exhibit different or enhanced responses to certain chemicals than most persons exposed to the same level of these chemicals in the environment. Factors involved with increased susceptibility may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters can reduce detoxification or excretion or compromise organ function.

Populations at risk of exposure to glyphosate at unusually high levels are discussed in Section 5.7, Populations with Potentially High Exposures.

Limited information was located regarding possible age- or gender-related differences in susceptibility to toxic effects from glyphosate technical or glyphosate formulations. Panzacchi et al. (2018) added glyphosate or Roundup Bioflow® to the drinking water of rat dams from GD 6 through lactation and to their offspring up to postpartum day 125 at a concentration resulting in a dose of 1.25 mg glyphosate/kg/day. Microbiome profiling of the gut resulted in significant changes in overall bacterial composition in the pups only (particularly apparent prior to puberty); this effect was noted for glyphosate and for Roundup Bioflow®. Romano et al. (2010) employed Roundup Transorb® as test substance and found decreased serum testosterone in young male rats gavaged at a dose as low as 5 mg/kg/day; however, the effect may have been caused, at least in part, by other ingredients in the glyphosate formulation.

3.3 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as biomarkers of exposure, biomarkers of effect, and biomarkers of susceptibility (NAS/NRC 1989).

A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself, substance-specific metabolites in readily obtainable body fluid(s), or excreta. Biomarkers of exposure to glyphosate are discussed in Section 3.3.1. The National Report on Human Exposure to Environmental Chemicals provides an ongoing assessment of the exposure of a generalizable sample of the U.S. population to environmental chemicals using biomonitoring (see http://www.cdc.gov/exposurereport/). If available, biomonitoring data for glyphosate from this report are discussed in Section 5.6, General Population Exposure.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that (depending on magnitude) can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts formed by covalent bonding of a chemical to DNA, the formation of which can induce abnormal replication, mutation, and/or prevent proper DNA repair). Biomarkers of effect caused by glyphosate are discussed in Section 3.3.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.2, Children and Other Populations that are Unusually Susceptible.

3.3.1 Biomarkers of Exposure

Glyphosate and the metabolite, AMPA, have been measured in blood and urine (e.g., Connolly et al. 2018; Conrad et al. 2017; Zouaoui et al. 2013). However, most absorbed glyphosate is rapidly excreted as parent compound. Meaningful quantification of exposure would require analysis of blood and/or urine within hours following exposure.

3.3.2 Biomarkers of Effect

No information was located regarding biomarkers of effect specific to glyphosate toxicity.

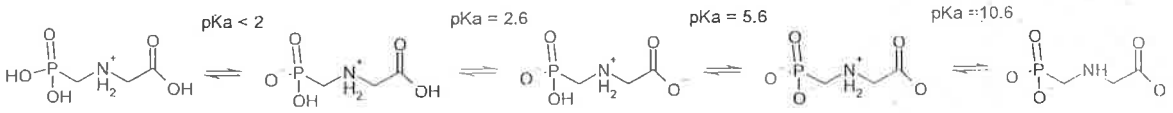
3.4 INTERACTIONS WITH OTHER CHEMICALS

Surfactants such as POEA in glyphosate-containing products might enhance the toxicity of glyphosate; results from one study indicate that the surfactant may be more acutely toxic than glyphosate or the combination of glyphosate and POEA (e.g., Adam et al. 1997).

CHAPTER 4. CHEMICAL AND PHYSICAL INFORMATION

4.1 CHEMICAL IDENTITY

Glyphosate is an organic acid composed of a phosphonomethyl and glycine component. The chemical name for glyphosate is *N*-(phosphonomethyl) glycine. Glyphosate is a zwitterion with four distinct dissociation constants (pKa values are depicted below) and exists as different ionic species depending on the pH of its surroundings. Glyphosate is an amphoteric chemical and may react as an acid or a base under certain conditions.



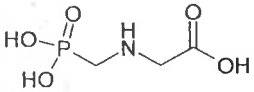
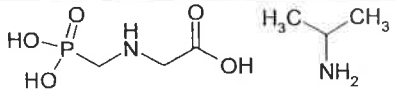
Glyphosate isopropylamine (Chemical Abstracts Registry Number [CASRN] 38641-94-0) is one of the salt forms of glyphosate used in commercial herbicides employing glyphosate as an active ingredient. This substance is registered as a pesticide by the EPA (1993) and is used to control broadleaf weeds and grasses; in food and nonfood settings, flower gardens, lawns, turf, residential areas, and forests; and along roadsides. Some labels may list the active ingredient a formulation of glyphosate and the acid equivalents (AE), which is the theoretical yield of the parent acid from the formulated ester or salt. For example, the AE of glyphosate isopropylamine salts is 74%.

Detailed information on the chemical identity of glyphosate and glyphosate isopropylamine is provided in Table 4-1.

4.2 PHYSICAL AND CHEMICAL PROPERTIES

Detailed information on the physical and chemical properties of glyphosate and glyphosate isopropylammonium is provided in Table 4-2.

Table 4-1. Chemical Identity of Glyphosate and Glyphosate Isopropylamine[a]

Characteristic	Information	
Chemical name	Glyphosate	Glyphosate isopropylamine
Synonym(s)	Glyphosphate; N-(phosphonomethyl) glycine; phosphonomethyliminoacetic acid; glyphosate acid	Glycine, N-(phosphonomethyl)-, compound with 2-propanamine (1:1); glyphosate-isopropylammonium; glyphosate mono(isopropylamine) salt; glyphosate-mono(isopropylammonium); N-(phosphonomethyl)glycine, isopropylamine salt
Partial list of registered trade name(s)	Pondmaster; Roundup® Max; Glifoglex; Glycel; Muster; Rondo; Sonic; Spasor; Sting; Tumbleweed; MON-0573; CP 67573	Roundup®; Rondo; Rodeo; Glifonox; Glycel; MON-0139; CP 70139; Shackle[b]
Chemical formula	$C_3H_8NO_5P$	$C_3H_8NO_5P.C_3H_9N$
Chemical structure		
CAS Registry Number	1071-83-6	38641-94-0

[a]All information obtained from McBean (2011), O'Neil et al. (2013), and/or ChemIDplus (2017) unless noted otherwise.
[b]EPA 1993.

CAS = Chemical Abstracts Service

Table 4-2. Physical and Chemical Properties of Glyphosate and its Isopropylamine Salt[a]

Property	Glyphosate	Glyphosate isopropylamine salt
Molecular weight	169.1	228.2
Color	White	White
Physical state	Solid; crystals	Powder
Melting point	230°C (decomposes)	Two stages: 143–164 and 189–223°C
Boiling point	No data	Decomposes without boiling
Density at 20°C	1.705	1.482
Odor	Odorless	Odorless
Odor threshold:		
Water	No data	No data
Air	No data	No data
Solubility:		
Water at 25°C	12,000 mg/L 10,500 mg/L (pH 1.9, 20°C)	1,050,000 mg/L (pH 4.3, 25°C)
Organic solvent(s)	Insoluble in most organic solvents: acetone, ethanol, and xylene	Dichloromethane 184 mg/L at 20°C; methanol 15,880 mg/L at 20°C
Dissociation constants:	pKa_1 0.8; pKa_2 3; pKa_3 6; pKa_4 11; pKa_1[b] <2; pKa_2[b] 2.6; pKa_3[b] 5.6; pKa_4[b] 10.6	pKa_1 2.18 at 20°C (monophosphate); pKa_2 5.77 at 20°C (carboxylic acid)
Partition coefficients:		
Log K_{ow}	<-3.4	-5.4
Log K_{oc}	3.4–3.7 (K_{oc}=2,600–4,900)[c]	No data
Vapor pressure at 25°C	9.8×10^{-8}	1.58×10^{-8}
Henry's law constant	2.1×10^{-12} atm-m^3/mol at 25°C[d]	3.3×10^{-15} atm-m^3/mol at 25°C[d]
Autoignition temperature	No data	No data
Flashpoint	Not flammable	No data
Flammability limits	No data	No data
Explosive limits	No data	No data

[a]All information obtained from either McBean (2011) or O'Neil et al. (2013).
[c]Glass 1987.
[b]Sprankle et al. 1975.
[d]EPI Suite 2012.

CHAPTER 5. POTENTIAL FOR HUMAN EXPOSURE

5.1 OVERVIEW

Glyphosate has not been identified in any of the 1,832 hazardous waste sites that have been proposed for inclusion on the EPA National Priorities List (NPL) (ATSDR 2015). However, the number of sites evaluated for glyphosate is not known.

- Occupational and residential exposure is a result of glyphosate's use in agricultural, non-agricultural, industrial, and residential settings. The highest potential for dermal, inhalation, and ocular exposure is expected for pesticide applicators, farm workers, and home gardeners who use herbicides containing glyphosate.
- The general population is exposed to glyphosate via ingestion of crops, plants, and foods with residues of this chemical. Residential exposure may occur via inhalation, dermal contact, and/or ocular contact during mixing or application of consumer products containing glyphosate or by coming into contact with crops, soils, or water to which glyphosate-containing products have been applied.
- Occupational exposure to glyphosate may occur via inhalation, dermal contact, and/or ocular contact during manufacture, transport, mixing, loading, application, and disposal processes. Accidental oral exposure may occur via unintentional ingestion. Dermal contact appears to be the major route of exposure to glyphosate for individuals involved in its application.
- Glyphosate mainly enters the environment as a direct result of its herbicidal use. Fate of this chemical in the environment includes degradation, transport, and partitioning processes, which are governed by its physicochemical properties and by abiotic or biotic degradation under certain environmental conditions. Glyphosate is a nonvolatile, highly polar, non-residual herbicide that has low potential for environmental persistence and is unlikely to bioaccumulate.

5.2 PRODUCTION, IMPORT/EXPORT, USE, AND DISPOSAL

5.2.1 Production

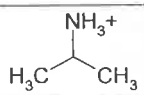
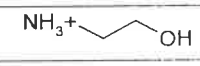
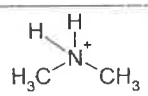
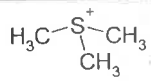
No information is available in the Toxics Release Inventory (TRI) database on facilities that manufacture or process glyphosate because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2005b).

Production of glyphosate is achieved through heating phosphorous acid and *a*-amino acetic acid followed by the addition of formaldehyde (Muller and Applebyki 2010). Glyphosate may also be produced by heating glycine and chloromethylphosphonic acid in aqueous sodium hydroxide (IPCS 1994).

Glyphosate is produced commercially in the United States as a technical-grade substance with a purity ≥95% (McBean 2011).

Glyphosate is typically manufactured for commercial use as a salt available in soluble liquid and soluble granule formulations. Salt forms of glyphosate include the isopropylamine salt, sodium salt, and monoammonium salt. Table 5-1 summarizes some of the common glyphosate salts that may be used as active ingredients in herbicides. Due to the various salt forms, the active ingredient listed on products is sometimes expressed in terms of acid equivalent.

Table 5-1. Glyphosate Salts

Name	CAS Registry Number	EPA PC Code	Cation	U.S. registration[a]
Glyphosate isopropylamine salt	38641-94-0	103601		Yes
Glyphosate mono ammonium	40465-66-5	103604	NH_4^+	Yes
Glyphosate ethanolamine salt	40465-76-7	103605		Yes
Glyphosate triammonium salt	114370-14-8	103607	NH_4^+	Yes
Glyphosate diammonium salt	69254-40-6	103607	NH_4^+	Yes
Glyphosate dimethylammonium salt	34494-04-7	103608		Yes
Glyphosate potassium salts	70901-12-1; 70901-20-1; 39600-42-5	103613	K^+	Yes
Glyphosate monosodium salt	34494-03-6	103603	Na^+	No
Glyphosate sesquisodium salt	70393-85-0	103603	Na^+	No
Glyphosate trimesium	81591-81-3	128501		No

[a]Pan 2014

CAS = Chemical Abstracts Service; EPA = U.S. Environmental Protection Agency; PC = pesticide chemical

Herbicide formulations employing glyphosate salts are commonly produced in combination with additives, inert ingredients, and surfactants. The salt derivatives enhance absorption of glyphosate from the surface of the plant or leaf structure, but are not the herbicidally active portion of the compound. Specific formulations vary in composition and are marketed under numerous trade names (NPIRS 2017; PAN 2009). Polyoxyethylene amine (POEA) (CASRN 24911-53-5) is a surfactant used in the

commercial product Roundup® (PAN 2009). Surfactants are used in herbicide formulations to increase penetration of glyphosate into plants. Sulfuric acid (CASRN 7664-93-9), phosphoric acid (CASRN 7664-38-2), propylene glycol (CASRN 57-55-6), and sodium benzoate (CASRN 532-32-1) are examples of additives used in some formulations (IPCS 1994; PAN 2009). Products may contain other active ingredients such as simazine (CASRN 122-34-9) and 2-methyl-4-chlorophenoxyacetic acid (CASRN 94-74-6). The herbicide 2,4-dichlorophenoxyacetic acid (CAS 94-75-7) may also be present at concentrations ranging from 11.1 to 20.6% (IPCS 1994). Commercial products containing glyphosate have been reported with concentrations ranging from 0.96 to 94 w/w%. The common herbicide, Roundup®, has product formulations containing glyphosate concentrations ranging from 0.96% to 71% (w/w) (NPIRS 2017; PAN 2016b). These products may be diluted depending upon the labeled use as per manufacturers specifications.

The introduction of glyphosate-resistant crops such as soybeans in 1996, canola and cotton in 1997, and maize in 1998, along with the distribution of their genetically engineered seeds, had major impacts on the production and demand for glyphosate.

According to the National Pesticide Information Retrieval System (NPIRS), as of May 2017, there were 43 companies manufacturing EPA federally registered products under the active pesticide code 417300 (glyphosate) (since many chemical names are too long to be handled easily, EPA assigns a 6-digit chemical code number for every active chemical ingredient), which are available for use in the United States; see Table 5-2 (NPIRS 2017). In addition, there were 72 companies in the United States that were manufacturing chemicals under the active pesticide code 103601 (glyphosate isopropylamine salt) (NPIRS 2017).

Table 5-2. Companies Manufacturing Products Under Pesticide Code 417300 (Glyphosate)

Company	Address	City, State, Zip Code
Syngenta Crop Protection, LLC	410 Swing Road	Greensboro, North Carolina 27419
The Scotts Company	D/B/A The Ortho Group, 14111 Scottslawn Road	Marysville, Ohio 43041
FMC Corporation, Agricultural Products Group	1735 Market Street	Philadelphia, Pennsylvania 19103
Monsanto Company	Chesterfield Village Research Center, 700 Chesterfield Parkway North	Chesterfield, Missouri 63017
Winfield Solutions, LLC	P.O. Box 64589	St. Paul, Minnesota 55164

Table 5-2. Companies Manufacturing Products Under Pesticide Code 417300 (Glyphosate)

Company	Address	City, State, Zip Code
ABC Compounding Co., Inc.	P.O. Box 16247	Atlanta, Georgia 30321
Cheminova A/S	P.O. Box 9	DK-7620 Lemvig
Helena Chemical, Co.	225 Schilling Boulevard, Suite 300	Collierville, Tennessee 38017
Chemsico, A Division of United Industries Corporation	P.O. Box 142642	St. Louis, Missouri 63114
Adama Agan Ltd	P.O. Box 262	Ashdod, 77102, Israel
Drexel Chemical Company	P.O. Box 13327	Memphis, Tennessee 38113
Loveland Products, Inc.	P.O. Box 1286	Greeley, Colorado 80632
Nufarm Limited	103–105 Pipe Road	Laverton North, Victoria 3026 Australia
Albaugh, LLC	P.O. Box 2127	Valdosta, Georgia 31604
Atanor S.A.	Foreign Trade Department, Albarellos 4914	B1605 AFR, Munro, Providence de Buenos Aires
BASF Sparks, LLC	P.O. Box 13528	Research Triangle Park, North Carolina 27709
Control Solutions, Inc.	5903 Genoa-Red Bluff Road	Pasadena, Texas 77507
Tenkoz, Inc.	1725 Windward Concourse	Alpharetta, Georgia 30005
Dow AgroSciences, LLC	9330 Zionsville Rd 308/2e	Indianapolis, Indiana 46268
Makhteshim Agan of North America, Inc.	d/b/a Adama, 3120 Highwoods Boulevard, Suite 100	Raleigh, North Carolina 27604
United Phosphorus, Inc.	630 Freedom Business Center, Suite 402	King of Prussia, Pennsylvania 19406
Monsanto Company	Lawn & Garden Products, 600 13th Street, NW, Suite 660	Washington, DC 20005
Helm Agro US, Inc.	401 E. Jackson Street, Suite 1400	Tampa, Florida 33602
Mey Corporation	121 South Estes Drive, Suite 101	Chapel Hill, North Carolina 27514
Sharda Cropchem, Limited	Domnic Holm, 29th Road	Bandra (West), Mumbai 400050
Rotam Agrochemical Company, Ltd.	26/F, E-Trade Plaza, 24 Lee Chung Street	Chaiwan, Hong Kong
Sharda USA LLC	P.O. Box 640	Hockessin, Delaware 19707
Ragan and Massey, Inc.	101 Ponchatoula Parkway	Ponchatoula Louisiana 70454
Tide International, USA, Inc.	21 Hubble	Irvine, California 92618
Agsaver II, LLC	P.O. Box 111	McGehee, Arkansas 71654
Repar-Glypho, LLC	8070 Georgia Avenue, Suite 209	Silver Spring, Maryland 20910
Farmway, Inc.	P.O. Box 640	Hockessin, Delaware 19707
Consus Chemicals, LLC	22 Pine Tree Drive	Wayne, New Jersey 07470
Axss Technical Holdings, LLC	111 Martin Road	Fulton, Mississippi 38843
Cinmax International, LLC	3050 Suite 113	Bloomington, Minnesota 55425

Table 5-2. Companies Manufacturing Products Under Pesticide Code 417300 (Glyphosate)

Company	Address	City, State, Zip Code
Agromarketing Co., Inc.	133 Mavety Street	Toronto, Ontario, Canada M6P
Glysorttech, LLC	281 Hampshire Drive	Plansboro, New Jersey 08536
Liberty Crop Protection, LLC	4850 Hahns Peak Drive, Suite 200	Loveland, Colorado 80538
Gly-Peak, LLC	224 South Bell Avenue	Ames, Iowa 60010
Tundra Agroindustrial, Ltd.	P.O. Box 10	Lemars, Iowa 51031
Argustoli H.C., LLC	10191 Park Run Drive, Suite 110	Las Vegas, Nevada 89145
Genmerica NA LLC	P.O. Box 1603	Cheyenne, Wyoming
Gruhn Mill Crop Solutions, LLC	701 Fifth Avenue, Suite 6100	Seattle, Washington 98104

Source: NPIRS 2017

5.2.2 Import/Export

No information was found concerning U.S. imports and exports of glyphosate.

5.2.3 Use

Glyphosate is a phosphonoglycine herbicide, first registered for use by the EPA in 1974. In June 1986, glyphosate was issued a Registration Standard (EPA 1986c) requiring additional data, which included phytotoxicity, environmental fate, toxicology, product chemistry, and residue chemistry studies; reregistration of single active ingredient formulations, plus one additional active ingredient formulation, were finalized in 1993 (EPA 1993). Glyphosate is registered for pre- and post-emergent applications for weed control in the production of various fruit, vegetable, and field crops. Glyphosate may be applied to fields prior to planting in order to remove unwanted weeds and vegetation or in preparation for harvesting in glyphosate resistant crops. Recommended application rates, methods of application and timing, temperature considerations, etc. may be found on individual product labels. Glyphosate is in the process of registration review by EPA; docket ID: EPA-HQ-OPP-2009-0361-0066 (EPA 2017c).

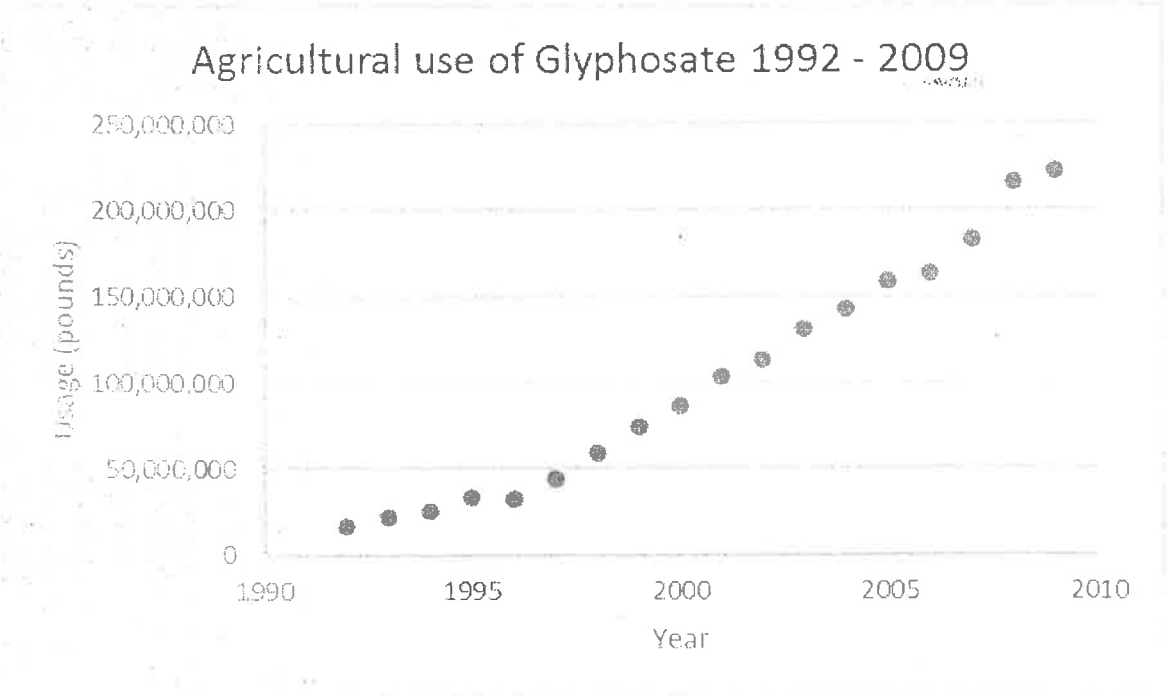
Glyphosate is used as a non-selective contact herbicide. Formulations are applied directly to control native and invasive weeds and vegetation around food crops and non-food field crops, and in non-crop areas such as roadsides, golf courses, right-of-way locations, and aquatic areas. Glyphosate is used in agriculture, forestry, industrial, lawn and garden, and aquatic (e.g., Rodeo®, Clearcast®) environments for weed control. In aquatic usage, the formulation typically contains no surfactant or a surfactant that is

nontoxic to aquatic organisms and applications must be made as per the product instructions to avoid rapid vegetative decay, which can lead to anaerobic environments and potential fish kills (Dow 2017). Glyphosate is applied to control broad-leaved weeds and woody brush, as well as annual and perennial grasses (Muller and Applebyke 2010; Plimmer et al. 2004). The sodium salt (CASRN 34494-03-6) can be used as a plant growth regulator for peanuts and sugarcane (EPA 1993). Glyphosate is a foliar-applied herbicide. Before the introduction of genetically modified glyphosate-resistant crops, application generally occurred before crops were planted (Duke and Powles 2008). After successful production and approval of glyphosate-resistant crops, such as soybean, cotton, maize, and canola, application generally occurs after planting and before harvest; the timing depends on the specific application (Duke and Powles 2008; Muller and Applebyke 2010). The introduction of these glyphosate-resistant crops increased the use of herbicidal products containing this chemical because it is possible to use it post-emergence without actually harming the crop. Greater than 90% of the soybeans produced in the United States are glyphosate tolerant, and most cotton (72%) and about half of the corn (52%) planted in 2007 were glyphosate tolerant (Coupe et al. 2012). It has been estimated that genetically engineered glyphosate-tolerant crops now account for about 56 % of its global usage (Benbrook 2016). Application techniques include aerial treatments, typically used for large-scale purposes, and wiping equipment or spraying equipment attached to vehicles, generally used for small-scale applications (FAO 1997; IPCS 1994).

According to data from the Pesticide Action Network (PAN) Pesticide Database, there are 102 products containing glyphosate (CASRN 1071-83-6) as the active ingredient, 94 of which have active registrations in the United States. There are 848 products containing glyphosate isopropylamine salt (CASRN 38641-94-0) as the active ingredient, of which 739 have active registrations in the United States (PAN 2016a, 2016b).

Increasing trends in annual agricultural use data for the United States are reflected from the use statistics available from the U.S. Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Program. Estimated yearly usage increased from approximately 20 to 60 million pounds from 1992 to 1998, from approximately 70 to 130 million pounds from 1999 to 2003, from approximately 140 to 250 million pounds from 2004 to 2011, and steady use of approximately 285–290 million pounds from 2012 through 2014 (USGS 2017). Figure 5-1 illustrates the agricultural use of glyphosate from 1992 to 2009 in the United States (USGS 2013).

Figure 5-1. Agricultural Application Trends of Glyphosate in the United States According to U.S. Geological Survey (USGS) Data



Source: USGS 2017

Benbrook (2016) compiled data from the National Agricultural Statistical Service (NASS) to estimate the amount of glyphosate applied for weed control in the production of major agricultural crops and non-agricultural (residential uses) in the United States from 1990–2014). The trends are summarized in Table 5-3.

Table 5-3. Glyphosate AI (Pounds) Usage Trends from 1990 to 2014

Crop	1990 Active ingredient (pounds)	2014 Active ingredient (pounds)	% Increase
Soybean	2,663,000	122,473,987	4,499.10%
Corn	880,066	68,949,452	7,734.58%
Cotton	192,429	17,421,787	8,953.62%
Wheat (winter)	331,758	12,353,488	3,623.64%
Alfalfa	381,525	8,853,600	2,220.58%
Sorghum	236,305	4,178,573	1,668.30%
Sugar beets	36,130	2,763,075	7,547.59%
Canola	0	219,392	NA
Wheat spring	75,308	1,201,807	1,495.86%

Table 5-3. Glyphosate AI (Pounds) Usage Trends from 1990 to 2014

Crop	1990 Active ingredient (pounds)	2014 Active ingredient (pounds)	% Increase
Barley	13,1568	1,064,160	708.83%
Other cops	1,897,522	4,526,043	138.52%
Total	7,683,070	249,906,307	3,152.69%
		Non-Agricultural Use	
	5,300,000	26,519,000	400.36%

Source: Benbrook 2016

The EPA recently granted the registration of a new herbicide named Enlist Duo™ containing 2,4-D choline salt and glyphosate for use on genetically modified corn and soybean crops designed to be resistant to 2,4-D and glyphosate (EPA 2014).

5.2.4 Disposal

Wastes resulting from products containing glyphosate should be disposed of at an approved waste disposal facility or in landfills approved for pesticide disposal. Disposal practices should be in accordance with federal, state, and local procedures. Non-refillable containers should never be reused. Empty containers should be rinsed thoroughly and offered for recycling, if available, or disposed of in accordance with container labels. Rinse-water can be emptied into formulation equipment and applied as residual pesticide in the appropriate manner. Do not contaminate fresh waters when disposing of equipment wash waters or container rinse waters. Containers that have not been completely rinsed may be considered hazardous and should be disposed of with regard to federal, state, and local regulations. Any unused product may be recycled by applying the product in an approved use setting or returning it to the manufacturer or supplier for safe disposal (Agrisolutions 2010; EPA 1993, 2011).

5.3 RELEASES TO THE ENVIRONMENT

TRI data should be used with caution because only certain types of facilities are required to report (EPA 2005b). This is not an exhaustive list. Manufacturing and processing facilities are required to report information to the TRI only if they employ ≥10 full-time employees; if their facility is included in Standard Industrial Classification (SIC) Codes 10 (except 1011, 1081, and 1094), 12 (except 1241), 20-39, 4911 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4931 (limited to facilities that combust coal and/or oil for the purpose of generating electricity for distribution in commerce), 4939 (limited to facilities that combust coal and/or

oil for the purpose of generating electricity for distribution in commerce), 4953 (limited to facilities regulated under RCRA Subtitle C, 42 U.S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited S.C. section 6921 et seq.), 5169, 5171, and 7389 (limited to facilities primarily engaged in solvents recovery services on a contract or fee basis); and if their facility produces, imports, or processes ≥25,000 pounds of any TRI chemical or otherwise uses >10,000 pounds of a TRI chemical in a calendar year (EPA 2005b).

No information is available in the TRI database on facilities that manufacture or process glyphosate because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2005b).

The use of glyphosate as an herbicide for crops and non-crop applications is the major source of glyphosate that intentionally enters the environment. Some glyphosate may be released from the manufacture, transport, and disposal of glyphosate or glyphosate-containing products. The majority of herbicidal formulations with glyphosate are directly applied to weeds to remove unwanted vegetation in residential and agricultural settings. Depending on its application, glyphosate may enter aquatic environments through direct application to control aquatic weeds (Dow 2017) or as a result of overspray in areas near aquatic environments. Aerial applications of glyphosate may result in unintended transport, depending on application technique and meteorological conditions, such as wind drift (EPA 1993; IPCS 1994; PAN 2009; Yates et al. 1978).

5.3.1 Air

There is no information on releases of glyphosate to the atmosphere from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005b).

Glyphosate released to the air from aerial and ground equipment has the potential for downwind transport. Yates et al. (1978) assessed the loss due to drift after application. The lowest drift losses resulted when ground sprayers operating at low pressure were employed. The highest drift losses occurred when jet nozzles were employed during aerial application performed by helicopter.

The Air Quality System (AQS) database is EPA's repository of criteria air pollutants and hazardous air pollutants (HAPs), containing monitoring data from >2,600 monitoring sites across the United States. Glyphosate has not been included in the AQS ambient air monitoring data as of 2016 (EPA 2017a).

5.3.2 Water

There is no information on releases of glyphosate to water from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005b).

Glyphosate may enter surface water systems either directly as a result of its aquatic use or indirectly due to overspray near surface water. Aquatic applications of glyphosate are used to control invasive aquatic species such as water chestnut (*Trapa natans*) or other labeled weeds (EPA 2010); however, no quantitative data are available regarding how much glyphosate is applied to aquatic waterways in the United States. Glyphosate may also enter surface waters indirectly due to transport of residues in run-off or erosion events. The amount of glyphosate transported to nearby water bodies from runoff and erosion is dependent upon several factors, including the frequency, timing, and application rate of glyphosate to nearby areas, meteorological conditions (e.g., rainfall events and duration), and the characteristics of the soils in the treated areas. Hydrological factors such as input to the waterbody from overland flow as compared to subsurface infiltration also effect potential pesticide loadings. Coupe et al. (2012) studied the glyphosate levels at three locations located in the United States (South Fork River Basin, Iowa; Sugar Creek River Basin, Indiana; and Bogue Phalia Basin, Mississippi). The basins are located in agricultural areas dominated by soybean, corn, rice, and cotton (Mississippi only) production, but have differing climates and soil characteristics. Water samples collected from 2007 to 2008 at three sites located in the Bogue Phalia basin all had detectable levels of glyphosate and its degradation product, AMPA. Glyphosate concentrations at the sites ranged from 0.03 to 73 µg/L. Levels showed a distinctive seasonal pattern with lowest levels occurring in winter, followed by a steady increase into late fall, which coincided with seasonal application timings of glyphosate. Moreover, both glyphosate and AMPA loads into the basin were greater in 2008 as compared to 2007, which corresponded to a higher rainfall rate for that year. Approximately 59–72% of the water samples collected from the South fork River basin had detectable levels of glyphosate ranging from <0.02 to 5.7 µg/L. Higher glyphosate loadings as a percentage of usage into the Bogue Phalia Basin as compared to the South Fork River Basin is a result a higher overland flow in the basin (as compared to subsurface water infiltration) and the fact that the majority of soils in the Bogue Phalia Basin are characterized as heavy clay soils classified as hydrologic soil groups C and D, which have higher runoff potential than the predominant soil types in the South Fork River Basin.

Glyphosate levels in the Sugar Creek River Basin, Indiana were limited to measurements taken during two heavy rainfall storm events in which 2.6 and 5.7 cm of rain were recorded. Glyphosate levels ranged from 0.16 to 430 µg/L, with the highest level recorded during the heavier rainfall event.

Battaglin et al. (2005) discussed the occurrence of glyphosate in 51 streams in the Midwestern United States from pre-emergence, post-emergence, and harvest runoff samples. Maximum levels in runoff water ranged from 1.00 µg/L (pre-emergence runoff) to 8.7 µg/L in harvest season runoff samples. Glyphosate levels in surface water are summarized in Section 5.5.2.

5.3.3 Soil

There is no information on releases of glyphosate to soil from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005b).

Glyphosate applied directly to vegetation may migrate to the soil from foliar washoff or translocation from the plants to the root zone. As discussed in Section 5.2.3, glyphosate agricultural uses in the United States increased from about 20 million pounds in 1992 to about 300 million pounds by 2014 (USGS 2017). Battaglin et al. (2014) estimated that nonagricultural uses of glyphosate were about 9,300 metric tons (20.5 million pounds) in the United States in 2007 and Benbrook (2016) estimated that about 26.5 million pounds were used for nonagricultural purposes in 2014.

A 2008 survey of pesticide application in Ontario, Canada, conducted by the Ministry of Agriculture, Food, and Rural Affairs reported that glyphosate use increased from 1,170,762 kg active ingredient in 2003 up to 2,062,648 kg active ingredient in 2008 (OMAFRA 2008). A total of 527,952 kg of glyphosate were used on field crops, 6,700 kg were used on fruit, 6,110 kg were used on vegetables, and 6,635 kg of glyphosate were used on nursery crops, sod, and ginseng; greenhouse crops were not included. Specific 2008 glyphosate applications for weed control by crop use amounted to 527,952 kg in production of field corn, 1,253,773 kg for soybean production, 11,087 kg for canola, 155,428 kg for wheat, 9,206 kg for oats, 6,588 kg for barley, 6,167 kg for mixed grains, 3,185 kg for rye, 18,054 kg for white beans, 18,661 kg for dry beans, 27,011 kg for hay, 2,717 kg for pasture, 1,386 kg for sugar beets, and 1,991 kg for other field crops (OMAFRA 2008).

A 2013/2014 survey of pesticide application in Ontario, Canada, conducted by the Ministry of Agriculture, Food, and Rural Affairs reported pesticide use for glyphosate (OMAFRA 2015). A total of

2,909,184 kg of glyphosate were used on all surveyed field crops in 2013/2014; 13,194 kg were used for fruit and 9,869 kg were used for vegetables. Specific crop use in 2013 for the amount of the active ingredient glyphosate applied as an herbicide equaled 1,151,051 kg for field corn, 1,544,954 kg for soybeans, 65,230 kg for wheat, 34,573 kg for oats and mixed grains, 11,542 kg for white beans, 27,980 kg for hay and pasture, and 24,144 kg for other field crops (OMAFRA 2015).

5.4 ENVIRONMENTAL FATE

The environmental fate of glyphosate, which includes the transport, partitioning, and transformation of this substance, is controlled by various physicochemical properties, degradation, and other loss processes. Glyphosate is a non-volatile, highly polar, non-residual herbicide that has low potential for environmental persistence and is unlikely to bioaccumulate; the chemical is either degraded or inactivated by adsorption to soil (Smith and Oehme 1992). Microbial degradation in soils and water is an important fate process; reported half-lives range from 2 to 215 days in soils and from 1.5 to 130 days in waters (Battaglin et al. 2014; IPCS 1994; PAN 2009; Rueppel et al. 1977). The wide range of half-lives is a result of environmental conditions such as soil characteristics, pH, and endogenous microbial populations, which are factors that influence the rate of degradation. Glyphosate is not expected to be susceptible to hydrolysis; photodegradation has not been confirmed as an important fate process in any environmental media (Smith and Oehme 1992).

5.4.1 Transport and Partitioning

Glyphosate is not expected to change ionic form at pH levels of 5–8 and is expected to exist in its anionic form under most environmental conditions.

Air. Glyphosate has a low vapor pressure and is expected to exist in the particulate phase in the ambient atmosphere. There is potential for spray drift after application of herbicides, the extent of which is dependent on the mode of application. Aerial applications may result in considerable transport depending on climate conditions (IPCS 1994; Yates et al. 1978). Drift analysis has shown that 10–37% of applied herbicide can drift to non-target plants. Seedling and plant fatalities were found 20–100 m downwind after application, and residues have been detected at 400 and 800 m downwind following ground and aerial applications, respectively (PAN 2009). Photolysis in air is not an important fate process (Rueppel et al. 1977). Particulate-phase glyphosate can be removed from the atmosphere by wet or dry deposition.

Wet deposition of glyphosate and its major degradation product, AMPA, from the atmosphere ranged from 3.9 to 16 μg/m^2 and from 1.7 to 5.2 μg/m^2, respectively, as reported in a study conducted in Pace, Mississippi, and Blairsburg, Iowa in 2007 and 2008 (Chang et al. 2011). In a study conducted in 2001, the total annual deposition for glyphosate was reported as 49,000 ng/m^2 and the maximum concentration detected was 6,200 ng/L. The total annual deposition for AMPA was reported as 12,757 ng/m^2 and the maximum concentration detected was 1,200 ng/L. The majority of glyphosate detections occurred during the spraying season. Deposition rates and concentrations of glyphosate were higher at the urban sites; this was attributed to its non-agricultural uses. The concentration of glyphosate and several other herbicides/pesticides were monitored in rainwater in Belgium from 1997 to 2001 (Quaghebeur et al. 2004). Glyphosate was detected in about 10% of the samples collected in 2001 at a maximum level of 11,000 ng/L.

Water. Depending on its application, glyphosate may enter aquatic environments through direct application or as a result of overspray in areas near aquatic environments. There is evidence of limited run-off and leaching with sandy soils and heavy rainfall (Borggaard and Gimsing 2008). Partitioning into aqueous environments is attenuated by adsorption to soils and sediments.

Sediment and Soil. Glyphosate will have strong adsorption to most soils due to its ionic nature and is expected to bind to positively charged metal surfaces present in clay and soils. Adsorption occurs through hydrogen bonding ion exchange or complexes of the phosphonate anion as well as the ammonium cation with minerals present in soils (Miles and Moye 1988). In an unpublished report by Monsanto in 1978, <0.1–6.6% of applied activity was recovered in the solution that washed off of the soil columns under leaching conditions simulating a heavy rainfall (IPCS 1994). The potential for run-off and leaching ability of glyphosate was examined by Rueppel et al. (1977) in three soils. Using inclined soil beds and artificial rainfall scenarios, a maximum runoff off <2x10^{-4} kg/ha was reported. Using thin layer chromatography and beta camera analysis, 97–100% adsorption to all three soils indicated that there is minimal possibility for leaching into groundwater. Although glyphosate is expected to adsorb strongly to soil particles and clay minerals, desorption may occur under certain conditions. It has been demonstrated that sorption decreases with increasing soil pH, increasing concentrations of inorganic soil phosphate, and decreasing mineral concentrations (Glass 1987; Gerritse et al. 1996; Piccola et al. 1994; Plimmer et al. 2004; Smith and Oehme 1992; Sprankle 1975). However, because of the strong sorption to most soils, mobility and the potential for migration into groundwater are low. The major degradation product, AMPA (CASRN 1066-51-9), also binds to soils and may be more mobile than glyphosate (Duke and

Powles 2008; IPCS 1994). Leaching of glyphosate may be possible under certain environmental conditions; however, it is not expected to leach into groundwater under most environmental conditions.

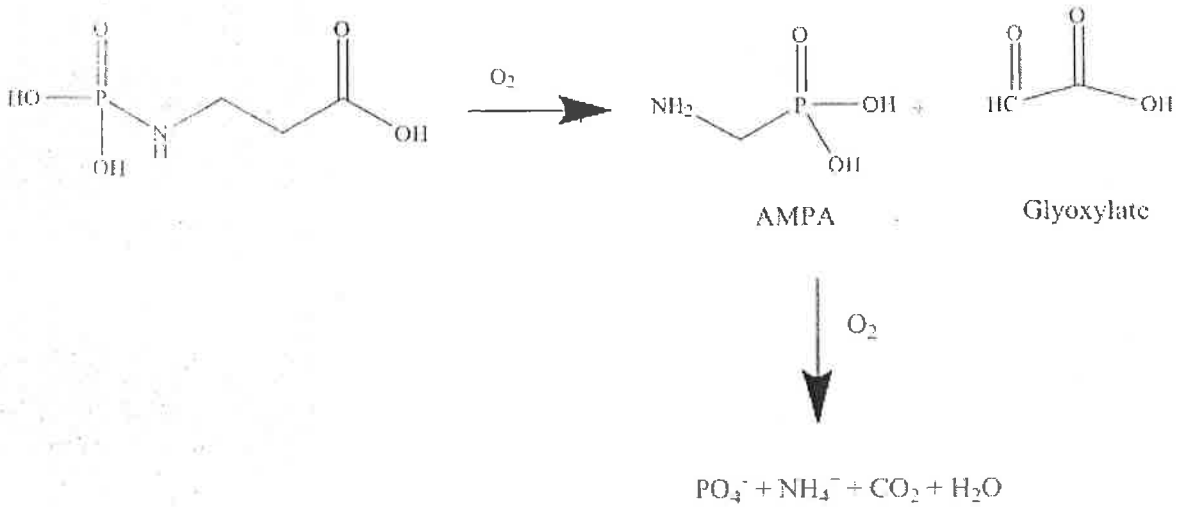
Other Media. Glyphosate is not generally taken up from the soil by a plant's root system since it typically forms bound residues with organic matter in most soils. Absorption of glyphosate via the roots has been discussed in a review by Saunders and Pezeshki (2015); however, many of the studies cited were conducted under hydroponic conditions, which are not likely to be typical of field environments. Some uptake has been demonstrated to occur under field conditions with low organic-containing soils. The EPA Registration Eligibility Decision (RED) document for glyphosate showed that lettuce, carrots, and barley contained glyphosate and AMPA residues after a sandy loam containing 0.3–0.5% organic matter was treated with 3.71 pounds of glyphosate per acre, but accumulation decreased as the length of rotation increased. For example, glyphosate levels were 0.097 ppm in lettuce planted 30 days post-treatment, but only 0.037 ppm in lettuce planted 119 days post-treatment (EPA 1993). After surface application of glyphosate, it may move from the point of application, typically the leaves, to other parts of the plant. Glyphosate can be absorbed into the plant or vegetable through its outer wall or skin and can move throughout the stem and leaves of the entire plant. Metabolism of glyphosate within the plant occurs slowly (Doublet et al. 2009; Smith and Oehme 1992; WHO 2005). Glyphosate is mobile inside the plant and may be transported within the phloem system into other tissues before the plant is killed (Duke and Powles 2008; Pankey 2000; Plimmer et al. 2004). Boerboom and Wyse (1988) investigated absorption and translocation of glyphosate using Canada thistle seeds with various concentrations of a formulation of glyphosate (356 g/L) and the surfactant POEA (178 g/L). Translocation from the treated leaf to the root was clearly observed. Translocation generally decreased as the concentration of glyphosate increased. Application of the smaller droplets resulted in greater translocation to the roots compared to application of larger droplets.

5.4.2 Transformation and Degradation

Glyphosate is readily and completely degraded in the environment mainly by microbial processes. Modes of degradation involving glyphosate oxidoreductase (GOX) and C-Plyase enzymatic pathways have been suggested. AMPA has been identified as the major metabolite in both soils and water. Sarcosine is an additional degradation product produced by the C-Plyase enzymatic pathway. Glyoxylic acid (CASRN 298-12-4) is an additional degradation product by the GOX enzymatic pathway. Both pathways result in complete mineralization to inorganic phosphate, carbon dioxide, ammonium, and water (Balthazor and Hallas 1986; Kishore and Jacob 1987; Shinabarger and Braymer 1986). AMPA has reported soil half-

lives ranging from 60 to 240 days and aquatic half-lives similar to glyphosate (Battaglin 2014). Figure 5-2 illustrates the degradation of glyphosate under aerobic conditions.

Figure 5-2. Degradation of Glyphosate Under Aerobic Conditions



Source: Schuette 1998

The high water solubility, low log K_{ow}, and ionic nature of glyphosate suggest that this compound would not be expected to bioaccumulate in aquatic organisms (IPCS 1994; WHO 2005). Jackson et al. (2009) measured whole-body bioconcentration factor (BCF) values for glyphosate in bluegill fish (*Lepomis macrochirus*) using EPA guideline method OPPTS 850.1730 for an exposure period of 28 days. A BCF value of 0.52 (log BCF -0.284) was reported, suggesting that bioconcentration was low. Accumulated residues of glyphosate in fish, crustaceans, and mollusks exposed to water containing glyphosate declined approximately 50–90% over 14–28 days after removal from the glyphosate water into glyphosate-free water (WHO 2005). Bioaccumulation of glyphosate in blackworms (*Lumbriculus variegatus*), following soil application of glyphosate and a commercial formulation, was investigated (Contardo-Jara et al. 2009). BCF values after 4 days of exposure to concentrations of 0.05–5 mg/L of both 98% pure glyphosate and the formulation Roundup Ultra® were measured at 20°C (Contardo-Jara et al. 2009). BCF values based on the fresh weight of the worms ranged from 1.2 to 5.9; the BCF values for pure glyphosate at 0.05, 0.5, and 5.0 mg/L were approximately 2.9, 1.1, and 2.8, respectively and BCF values for Roundup Ultra® at 0.05, 0.5, and 5.0 mg/L were approximately 5.9, 3.8, and 2.7, respectively. The greater uptake of glyphosate from the Roundup Ultra® sample was attributed to the surfactant in the formulation, POEA.

The mechanism of action for glyphosate's herbicidal properties involves the inhibition of enzymes in the shikimate pathway. Specifically, the enzyme enolpyruvylshikimate-3-phosphate synthase is inhibited, creating a deficiency of enolpyruvylshikimate-3-phosphate and an abundance of shikimate. It has been suggested that the actual death of the plant is due to the disruption of plant processes regulated by the shikimate pathway essential to plant health and growth such as the primary biosynthesis of aromatic amino acids like phenylalanine, tryptophan, and tyrosine, as well as lignin and chlorophyll, and secondary processes such as flavonoid synthesis. These primary processes are exclusive to plants and some microorganisms and do not occur in any animals; therefore, the inhibition of enzyme production induced by glyphosate only affects species in the plant kingdom. It has also been suggested that the increased carbon flow to the shikimate pathway decreases carbon available for other essential photosynthetic processes (Muller and Applebyke 2010; Pankey 2000; Plimmer et al. 2004; Servaites et al. 1987).

In transgenic plants modified to be glyphosate tolerant, glyphosate is converted to N-acetylglyphosate (CASRN 129660-96-4), a chemical that lacks herbicidal properties (Pioneer 2006). This chemical may be further metabolized to N-acetyl (aminomethyl)phosphonic acid (N-acetyl-AMPA) (PAN 2009).

Air. Glyphosate has low vapor pressure and is considered stable in ambient air. Photolysis in air was examined by Rueppel at al. (1977). Loss of ^{14}C-labelled glyphosate was <3% after 48 hours; therefore, direct photolysis is not an important fate process (48 hours of direct irradiation is similar to 16 8-hour days of sunlight).

Water. Glyphosate has high water solubility and is expected to exist as an anion at neutral pH (IPCS 1994; O'Neil et al. 2013). Based on experimental adsorption coefficients ranging from 8 to 377 dm^3/kg for various soil and clay substrates, glyphosate is expected to adsorb to suspended solids and sediments in water. Precipitation from water has been suggested due to water-insoluble metal complexes with iron(III), copper(II), calcium, and magnesium that have been found; coordination occurs through the amine nitrogen, the carboxylic oxygen, and the phosphate oxygen (Subramaniam and Hoggard 1988). Photodegradation in water is not expected to be an important fate process for glyphosate under environmentally relevant conditions. Experimental half-lives of <28 days upon exposure to natural light at pH 5, 7, and 9 have been reported (IPCS 1994; Rueppel et al. 1977). No detectable photodegradation was observed in a study using sterile water and exposure to ultraviolet (UV) light or natural sunlight (Smith and Oehme 1992). Lund-Hoje and Friestad (1986) exposed glyphosate to UV light at 254 nm at 20°C in the laboratory and exposed 1% glyphosate solutions in deionized water, polluted water, and water with suspended sediments to natural sunlight (measured λ=295–385 nm) outside at temperatures ranging

from 20 to -5°C. Results indicated that photodegradation occurred faster in pure water as opposed to polluted water or water with sediments in which adsorption accounted for the majority of dissipated glyphosate. A photolytic half-life of 3–4 weeks was observed for glyphosate, at an initial concentration of 2,000 ppm in the deionized water exposed to UV light. A photolytic half-life of 5 weeks at 100 ppm was observed for glyphosate in deionized water, exposed to natural sunlight. The rate of hydrolysis is considered very slow. In a study at 35°C, glyphosate did not undergo hydrolysis in buffered solutions with a pH of 5, 7, or 9. Laboratory studies have reported a half-lives of >14 days in water and sediment under aerobic conditions and 14–22 days under anaerobic conditions for glyphosate (IPCS 1994). In an aqueous hydrolysis study at 25°C in buffered solutions of pH 5, 7, and 9, glyphosate was considered hydrolytically stable, with extrapolated half-lives beyond 3 years (EPA Undated).

Rapid dissipation of glyphosate in small forest ponds was observed as a result of sediment sorption and microbial degradation (Goldsborough and Beck 1989). Dissipation in three ponds, pH 5.0–7.7, resulted in half-lives of 1.5–3.5 days. After 38 days, glyphosate was not detected in any of the samples. AMPA concentrations were consistently low throughout the study.

Microbial degradation of glyphosate in water sediments has been investigated. AMPA has been identified as the major metabolite in water. Rueppel at al. (1977) performed non-sterile and sterile soil/water shake flask experiments to examine the degradation of glyphosate under aerobic and anaerobic conditions. The ^{14}C-labeled glyphosate samples used were between 94.8 and 98.1% pure. Ray silt loam, Norfolk sandy loam, and Drummer silty clay loam soil samples were used. In the sterile soil test, 1.0% degradation was achieved after 7 days; the report suggests that abiotic chemical degradation is not a likely fate process for glyphosate. In the non-sterile aerobic and anaerobic tests in Ray silt loam, carbon labeled glyphosate achieved 46.8--55.3 and 33.5–55.3% degradation, respectively, after 28 days, measured by applied ^{14}C as CO_2 evolution. In the non-sterile aerobic tests in Drummer loams, both fresh and bin-stored, carbon-labeled glyphosate achieved just over 40% and just under 20% degradation, respectively, after 28 days, measured by applied ^{14}C as CO_2 evolution. In the fresh Drummer loam and Ray loam samples, no lag phases were observed and the bulk of the degradation occurred by day 7, after which time, the rate of degradation declined. The slowing of degradation was attributed to adsorption to soil. In Ray silt loam and Drummer silty clay loam, dissipation of glyphosate reached 90% after 14 and 80 days, respectively, and half-lives were reported as 3 and 25–27 days, respectively. The results were similar at different concentrations of glyphosate. In the non-sterile aerobic test in Norfolk sandy loam, carbon-labeled glyphosate achieved <10% degradation after 28 days, measured by applied ^{14}C as CO_2 evolution, and 43% dissipation occurred after 112 days. A half-life of 130 days was reported for Norfolk soil. The

principle degradation product identified, AMPA, was confirmed in soil samples by nuclear magnetic resonance (NMR) imaging, mass spectral analysis, ion-exchange chromatography, and thin-layer chromatography. Minor degradation products identified included N-methylaminomethylphosphonic acid, glycine, N,N-dimethylaminomethylphosphonic acid, and hydroxymethylphosphonic acid, all of which were typically present at <1% (Rueppel et al. 1977). The metabolite, AMPA, achieved 16.1 and 34.8% degradation after 63 days in Drummer and Ray loams, respectively, measured by applied ^{14}C as CO_2 evolution.

Abiotic degradation was examined by Ascolani Yael et al. (2014) in aqueous solution in the presence of copper salts; results indicated that glyphosate interactions with metal ions in soils may catalyze degradation to AMPA. Further investigation was proposed.

Sediment and Soil. Glyphosate is readily degraded in the terrestrial environment by a variety of microorganisms. Bacteria, actinomycetes, fungi, and other soil microbes have the ability to degrade glyphosate. AMPA has been identified as the major metabolite in soil. Glyphosate may also be degraded in soil to sarcosine and inorganic phosphate. Photodegradation is not expected to be an important fate process in soil.

After application of Roundup® at about 2.0 kg/ha (acid equivalent of isopropylamine salt of glyphosate) to Carnation Creek watershed (10 km^2 study area), 50% of the glyphosate residues in soil dissipated after 45–60 days and 82–94% dissipated after 360 days (Feng et al. 1990a).

It has been demonstrated that inorganic phosphate present in soils may inhibit some microbial degradation of glyphosate (Kishore and Jacob 1987). Strains capable of using glyphosate as a sole carbon, nitrogen, or phosphorus source, thereby degrading glyphosate, include *Flavobacterium* sp. (Balthazor and Hallas 1986), which is known to degrade glyphosate in the presence of phosphate, *Pseudomonas* sp. PG2982 (Kishore and Jacob 1987; Shinabarger and Braymer 1986), *Arthrobacter atrocyaneus* (Pipke and Amrhein 1988), and *Rhizobium* spp. (Liu et al. 1991). Biodegradation may involve co-metabolism with other energy sources as well (Sprankle et al. 1975). Degradation products include AMPA and glyoxylic acid, which are subsequently degraded to inorganic phosphate, carbon dioxide, and ammonium. In addition, some bacterial degradation results in the production of sarcosine and inorganic phosphate (Borggaard and Gimsing 2008; Kishore and Jacob 1987; Liu t al 1991; Pipke and Amrhein 1988; Shinabarger and Braymer 1986).

Microbial degradation of bound and unbound glyphosate in several soils resulted in 17.4–45% ultimate degradation after 28 days; the highest degradation rate was observed in Conover sandy clay loam soil (Sprankle et al. 1975). The majority of the degradation was attributed to co-metabolic processes of soil microbes, with possible chemical degradation occurring.

In a biodegradation experiment with activated sludge, the bacterial strain, *Flavobacterium* sp., was identified as the microorganism metabolizing glyphosate to AMPA. This degradation was followed by complete mineralization of AMPA, using the enzyme phosphonatase, to carbon dioxide (CO_2), phosphate (PO_4^{3-}), ammonium (NH^{4+}), and water (H_2O) (Balthazor and Hallas 1986).

A variety of microorganisms are capable of degrading glyphosate. In one degradation pathway, the initial step involves cleavage of the carbon-phosphate bond to produce sarcosine and inorganic phosphate. This is followed by conversion of sarcosine to glycine and formaldehyde. *Pseudomonas* sp. PG2982 uses the enzyme, C-P lyase, to cleave the carbon-phosphate bond in glyphosate, producing sarcosine. This is followed by the cleavage of sarcosine into glycine and formaldehyde (Kishore and Jacob 1987; Shinabarger and Braymer 1986). Glycine and formaldehyde are metabolized in other biosynthesis processes, such as the oxidation of formaldehyde to carbon dioxide. Multiple strains in the bacterial family *Rhizobiaceae* have the ability to metabolize glyphosate. Liu et al. (1991) found that rhizobia bacterial cells took up close to 85% of available glyphosate within 30 minutes, after which time, the percentage began to decrease. Thin layer chromatography confirmed the presence of sarcosine and glycine as degradation products.

Doublet et al. (2009) studied the degradation of plant absorbed glyphosate in soils. Plants containing residues of glyphosate can enter the soils during crop cycling or harvesting. Degradation of glyphosate was different depending on the plant tissue in which it was absorbed. Mineralization rate constants (k (day^{-1})) ranged from 0.031 to 0.097 in the apex of oilseed rape and in the lamina of maize, respectively. It was noted that absorption of glyphosate in plants delayed degradation in soil.

Glyphosate is expected to adsorb strongly to soil particles and clay minerals; however, the amount of glyphosate sorbed decreases with increasing soil pH. Adsorption and desorption of glyphosate were examined using HPLC (Gerritse et al. 1996; Glass 1987; Piccola et al. 1994; Sprankle et al. 1975). Adsorption to agricultural soils and clay minerals and the effects of pH and cation saturation were examined by Glass (1987). The K_{oc} values were 4,900 for clay loam with pH 7.5 and organic content (OC) of 1.56%; 3,400 for silt loam with pH 5.8 and OC of 1.64%; and 2,600 for sandy loam with pH 5.6

and OC of 1.24%. The adsorption and desorption of glyphosate and the effects of soil characteristics in four various soil types were assessed (Piccolo et al. 1994). Some characteristics for the four soils follow: Sample A, pH 8.0 and 0.00 OC % (64.1% silt); sample B, pH 5.8 and 3.73 OC% (46.3% sand); sample C, pH 4.6 and 9.23 OC % (81.5% sand); and sample D, pH 8.3 and 0.45 OC % (82.4% silt). The greatest adsorption occurred in the soil with the highest concentrations of iron (4.74%) and aluminum (1.57) oxides (sample B); the greatest desorption occurred in the soil with lowest concentration of iron (0.18%) and aluminum (0.16%) oxides (sample A). The percent desorptions of glyphosate from the four soils were 81% in sample A, 15% in sample B, 72% in sample C, and 35% in sample D. A ligand exchange mechanism is hypothesized for the adsorption of glyphosate involving either the phosphonic component or the carboxylic component of this substance and adsorption to iron and aluminum sites (Benetoli et al. 2010; Piccola et al. 1994). The adsorption and desorption of both glyphosate and its metabolite, AMPA, were examined by Gerritse et al. (1996) using five soil types. K_{oc} values calculated for soil organic carbon ranged from 8.5 to $5x10^6$ after 1 day and from 45 to $>5x10^6$ after 1 week. The strongest adsorption occurred in the soil with the highest iron and aluminum content. The weakest adsorption occurred in the soil with the highest organic content. These results indicate that glyphosate has a notable affinity towards some soils, particularly with lower pH values and greater mineral content, and desorption occurs under certain environmental conditions especially as pH values increase and mineral concentrations decrease.

During a monitoring study with mixtures of Roundup® plus an additional herbicide, soil adsorption and desorption studies were performed on soils from Baton Rouge, Bridge City, and Hammond Louisiana (LaDOTD 1995). The Hammond soil with a pH <8 adsorbed >90% of the applied glyphosate. Adsorption values (Kf) were 8.7, 0.1, and 0.34 for Baton Rouge, Bridge City, and Hammond soils, respectively. Desorption values (Kd) were 355, 0.04, and 0.005 µg/g for Baton Rouge, Bridge City, and Hammond soils, respectively.

Greater than 90% of the glyphosate residues detected in forest soil samples (pH 4.20–5.28), where herbicides containing glyphosate had been sprayed, were found in the upper layers (depth of 0–15 cm) of the soils in both seasonally flooded and well-drained soils, indicating minimal leaching of glyphosate (Feng et al. 1990b).

Glyphosate dissipates from soil under certain environmental conditions. Half-life values between 3 and 174 days have been reported. In field experiments, dissipation from the soil due to run-off has been demonstrated (IPCS 1994). Landry et al. (2005) examined the leaching potential and mineralization of

glyphosate in vineyard soils by monitoring outdoor soil columns from May 2001 to May 2002. Bare and grass-covered soils with pH values ranging from 8.0 to 8.4 were studied. Sand, silt, and clay contents were 23.8–34.4, 36.5–39.6, and 29.1–36.9%, respectively, of the bare soils and 26.2–35.6, 34.2–41.3, and 29.6–32.5%, respectively, of grass-covered soils. An aqueous solution of herbicide containing 340 mg/L glyphosate was applied to both soil column surfaces. Effluents from the bare and grass-covered soils were collected weekly and after heavy precipitation to evaluate leaching of glyphosate and AMPA. Glyphosate was detected in 37% of the bare soil leachates and 27% of the grass-covered soil leachates. The highest concentrations measured from the bare soil leachate and grass-covered leachate were 17 and 2.7 µg/L, respectively. AMPA was detected in 90% (maximum concentration 9.4 µg/L) of the bare soil leachates and 41% (maximum concentration 3.5 µg/L) of the grass-covered soil leachates. Mineralization analysis was performed at 20°C for 42 days in both soils. In the grass-covered soil and bare soil, ^{14}C-labeled glyphosate achieved 46.5 and 43.5% CO_2 evolution after 42 days, respectively. Rapid degradation was observed with no lag phase; the highest rate of degradation occurred within the first 2 days. It was suggested that the initial rapid degradation was based on the degradation of free glyphosate and slowing rates of degradation were attributed to the degradation of adsorbed glyphosate.

Other Media. After application of herbicides, 30–97% of the applied glyphosate may be taken up by the plant by absorption from the treated leaves. Glyphosate-based formulations containing surfactants (and adjuvants) have a higher rate of absorption compared to glyphosate water solutions (Doublet et al. 2009). Surfactants in herbicide formulations aid in the adsorption and absorption of the active ingredient. Glyphosate is absorbed by plant foliage and transported or moved through the plant via phloem vessels; translocation patterns depend on the specific species of plant. Glyphosate enters these vessels slowly, but once inside, it becomes 'trapped' because of the pH within the vessels, which causes ionization (Gomes et al. 2014; IPCS 1994). Glyphosate may be degraded or metabolized in plants, AMPA is a notable degradation product (Duke 2011). An examination of the metabolism of glyphosate in soybean and canola suggest that some plants use a GOX enzyme for the conversion of glyphosate to AMPA. Degradation of glyphosate in glyphosate-resistant crops may give a better picture of the metabolic processes without interferences found in conventional crops. In transgenic plants modified to be glyphosate tolerant, glyphosate is converted to N-acetylglyphosate, which lacks herbicidal properties (Pioneer 2006). This chemical may be further metabolized to N-acetyl-AMPA (PAN 2009). Glyphosate and AMPA accumulate less in glyphosate-resistant crops than in conventional crops. Lower glyphosate and AMPA levels in glyphosate-resistant canola compared to conventional crops suggested that metabolism is more rapid in glyphosate-resistant canola (Duke 2011).

5.5 LEVELS IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to glyphosate depends, in part, on the reliability of supporting analytical data from environmental samples and biological specimens. Concentrations of glyphosate in unpolluted atmospheres and in pristine surface waters are often so low as to be near the limits of current analytical methods. In reviewing data on glyphosate levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

Table 5-4 shows the lowest limits of detection (LODs) that are achieved by analytical analysis in environmental media. An overview summary of the range of concentrations detected in environmental media is presented in Table 5-5.

Table 5-4. Lowest Limit of Detection Based on Standards[a]

Media	Detection limit	Reference
Air	0.01 ng/m^3	Chang et al. 2011
Drinking water	5.99 µg/L (ppb)	EPA 1990
Surface water and groundwater	Glyphosate and AMPA 0.02–0.10 µg/L 0.005 µg/L	Lee et al. 2002; USGS 2002 Ibanez et al. 2005
Soil and sediment	Organic soil =0.05 µg/g Mineral soil=0.02 µg/g Foliage=0.10 µg/g Sediment=0.03 µg/g Soil=0.005 µg/g	Thompson et al. 1989 Ibanez et al. 2005
Whole blood	15 ng/mL	Aris and LeBlanc 2011
Urine	0.09 ng/mL 0.1 ng/mL	Biagini et al. 2004 Jensen et al. 2016
Milk	10 µg/L (ppb)	Jensen et al. 2016
Crops and commodities	0.01 mg/kg	Alferness 1993

[a]Detection limits based on using appropriate preparation and analytics. These limits may not be possible in all situations.

AMPA = aminomethylphosphonic acid

Table 5-5. Summary of Environmental Levels of Glyphosate

Media	Low	High	For more information
Outdoor air (ng/m^3)	<0.01 (glyphosate) <0.01 (AMPA)	9.1 (glyphosate) 0.97 (AMPA)	Table 5-6
Surface water (ppb)	0.02	27.80	Table 5-8
Ground water (ppb)	0.01	2.2	Table 5-9
Drinking water (ppb)	Not detected		Table 5-9
Food (ppb)	0.078	5.47	Section 5.5.4, Other Media
Sediment	Not detected		Table 5-10

AMPA = aminomethylphosphonic acid

A study by the USGS evaluated 3,732 environmental samples across 38 states and the District of Columbia from several studies examining glyphosate in the environment; the samples were collected between 2001 and 2010 from 1,341 different sites, including groundwater; lakes, ponds, and wetlands; soil water; streams; large rivers; precipitation; ditches and drains; soil and sediment; and waste water treatment plant outfall (Battaglin et al. 2014). Glyphosate was detected in 39.4% of all the samples, with a median value of <0.02 µg/L and a maximum value of 476 µg/L. Its degradation product, AMPA, was detected in 55% of all the samples, with a median value of 0.04 µg/L and a maximum value of 397 µg/L. Groundwater (n=1,171) had the smallest percentage of detections, with 5.8% for glyphosate and 14.3% for AMPA. Glyphosate was detected in 53% of the 1,508 stream samples and AMPA was detected in 72%. Glyphosate was detected in 34% and AMPA was detected in 30% of the 104 small body water samples such as lakes and ponds. Out of 11 waste water treatment plant (WWTP) samples, glyphosate and AMPA were detected in 9.1 and 82%, respectively. Out of 85 precipitation samples, glyphosate was detected in 71% and AMPA was detected in 72%. Glyphosate was detected in 71% of the 374 ditch and drain samples, with a median value of 0.02 µg/L and a maximum value of 427 µg/L. Glyphosate was only detected without its degradation product, AMPA, in 2.3% of all of the samples; AMPA was detected without glyphosate in 17.9% of the samples. In 42.7% of all of the samples, neither analyte was detected. Several sites with multiple samples during the years 2001–2005 and 2006–2010 indicated that the detection frequency and median concentration of both glyphosate and AMPA had increased in the environment (Battaglin et al. 2014). The highest level of glyphosate was detected in soils and sediments. Out of 45 samples, glyphosate was detected in 91%, with a median value of 9.6 µg/kg and a maximum value of 476 µg/kg. AMPA was detected in 93.3% of 45 samples, with a median value of 18 µg/kg and a maximum value of 341 µg/kg.

5.5.1 Air

Ambient air monitoring data for glyphosate are compiled in Table 5-6.

Table 5-6. Outdoor Air Monitoring Data for Glyphosate

Location	Date	Median concentration (range) in ng/m^3	Notes	Reference
Agricultural ambient air; Mississippi	2007	Glyphosate: 0.48 (<0.01–9.1) AMPA: 0.06 (<0.01–0.49)	Glyphosate and AMPA detected in 19/22 air samples	Chang et al. 2011
	2008	Glyphosate: 0.24 (<0.01–1.5) AMPA: 0.02 (<0.01–0.09)	Glyphosate and AMPA detected in 27/27 and 19/27 air samples, respectively	
Agricultural ambient air; Iowa	2007	Glyphosate: 0.08 (<0.01–5.4) AMPA: 0.02 (<0.01–0.97)	Glyphosate and AMPA detected in 11/18 and 10/18 air samples	Chang et al. 2011
	2008	Glyphosate: 0.22 (<0.01–7.7) AMPA: 0.04 (<0.01–0.38)	Glyphosate and AMPA detected in 13/18 and 11/18 air samples	
Agricultural breathing zones; Baton Rouge, Bridge City, Hammond, Louisiana;	June 19, 1990–October 9, 1990	<0.1–138.6 µg/m	Breathing zone air (110 samples); sampled in areas where mixtures of commercial herbicides were applied using spray equipment with operating capabilities of 0.37 L/minute	LaDOTD 1995

AMPA = aminomethylphosphonic acid

5.5.2 Water

A comprehensive study conducted by the USGS from 2001 to 2006 examined glyphosate and its degradation products, glufosinate and AMPA, in 2,135 groundwater and surface water samples, 14 rainfall samples, and 193 soil samples in major river basins in the United States (USGS 2007). Results indicated that AMPA was detected more frequently and at similar concentrations than parent glyphosate in many samples, whereas glufosinate was seldom detected. The results are summarized in Table 5-7.

Table 5-7. Glyphosate and its Degradation Products in Water Samples in Major U.S. River Basins

	Glyphosate			AMPA			Glufosinate		
N	Detections	Maximum (µg/L)	Minimum (µg/L)	Detections	Maximum (µg/L)	Minimum (µg/L)	Detections	Maximum (µg/L)	Minimum (µg/L)
					Groundwater				
873	68	4.7	0.02	133	2.6	0.02	0	NA	NA
					Surface water				
1,262	489	427	0.02	725	41	0.02	7	1.5	0.05
					Rainfall				
14	12	1.1	0.3	12	0.47	0.02	0	NA	NA

Source: USGS 2007

Additional water monitoring data for glyphosate are compiled in Tables 5-8 and 5-9.

5.5.3 Sediment and Soil

Sediment and soil monitoring data for glyphosate are compiled in Table 5-10.

5.5.4 Other Media

In 2006, 20 prepared food samples were examined for glyphosate residues using electrospray ionization–liquid chromatography tandem mass spectrometry with limit of quantitation of 0.01 mg/kg and an LOD of 0.005 mg/kg (McQueen et al. 2012). Composite food samples assessed had a mean concentration of 0.08 mg/kg.

Four weeks post application of glyphosate at 4.5 kg/ha to separate pots planted with conventional corn, cotton, soybeans, and wheat, concentrations of glyphosate were 0.21, 0.26, 0.20, and 0.20 mg/kg, respectively. Six weeks after application, concentrations in corn, cotton, soybeans, and wheat were 0.14, 0.21, 0.29, and 0.18 mg/kg, respectively, and 8 weeks after application, concentrations in corn, cotton, soybeans, and wheat were 0.079, 0.42, 0.076, and 0.35 mg/kg, respectively (FAO 2005). Four-week concentrations of glyphosate in control crops of corn, cotton, soybeans, and wheat were 0.068, 0.04, 0.029, and 0.008 mg/kg, respectively. Six-week concentrations in control crops of corn, cotton, soybeans, and wheat were 0.089, 0.020, 0.11, and 0.015 mg/kg, respectively, and 8-week concentrations in control crops of corn, cotton, soybeans, and wheat were 0.022, 0.27, 0.045, and 0.061 mg/kg, respectively (FAO 2005).

Table 5-8. Surface Water Monitoring Data for Glyphosate

Location	Date	Concentration (range) in µg/L	Notes	Reference
Surface water United States	2016	Mean: 0.30 ; Median 0.10; (0.02–5.1)	EPA STORET data: Routine monitoring samples from USGS Science Centers in Arkansas, California, Colorado, Connecticut, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan Center, Maryland, Massachusetts, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Jersey, New Mexico, New York, North Carolina, North Dakota, Oregon, South Carolina, Texas, Utah, Washington, and Wyoming	WQP 2017
Surface water United States	2015	Mean: 0.27; Median 0.08; (0.02–24.20)	EPA STORET data: Routine monitoring samples from Minnesota Department of Agriculture--Pesticide and USGS Science Centers in Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan Center, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Jersey, New Mexico, New York, North Dakota, North Washington, Ohio, Oklahoma, Oregon, South Carolina, South Dakota, Texas, Utah, Washington, and Wyoming	WQP 2017
Surface water United States	2014	Mean: 0.38; Median 0.10; (0.02–8.10)	EPA STORET data: Routine monitoring samples from Minnesota Department of Agriculture--Pesticide and USGS Science Centers in Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Florida, Georgia, Hawaii, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, Texas, Utah, Virginia, Washington, and Wyoming	WQP 2017
Surface water United States	March to October 2013	Mean: 0.85; Median 0.34; (0.02–27.80)	EPA STORET data: Routine monitoring samples from Minnesota Department of Agriculture and USGS Science Centers in Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Minnesota, Missouri, Nebraska, New York, North Carolina, Ohio, South Dakota, Wisconsin, and Wyoming	WQP 2017

220

Table 5-8. Surface Water Monitoring Data for Glyphosate

Location	Date	Concentration (range) in µg/L	Notes	Reference
Rivers, small streams, agricultural ditches, and low flow wetlands Southern Ontario	May and mid-December 2004; April and November 2005	5–41	2004: 203 surface water samples collected from 26 sites 2005: 299 samples taken from 58 sites ~50% of sites detected glyphosate multiple times AMPA detected at trace levels (20–66 µg/L in 5.4% of samples)	Struger et al. 2008
Streams Minnesota, Wisconsin, Nebraska, Iowa, Illinois, Indiana, Ohio, Kansas, and Missouri	2002	Minimum: 0.10–0.46 detected in Iowa, Missouri, and Wisconsin Maximum: 0.54–8.7 detected in Illinois, Indiana, Kansas, Minnesota, Nebraska, and Wisconsin	51 locations (155 total samples); samples collected post-application of pre-emergence herbicides, post-application of post-emergence herbicides, and during the harvest season. Glyphosate detected at levels above the method reporting limit of 0.10 µg/L in 35% of pre-emergence samples, 40% of post-emergence samples, and 31% of harvest season samples. AMPA detected at levels >0.10 µg/L in 53% of pre-emergence samples, 83% of post-emergence samples, and 73% of harvest season samples.	Battaglin et al. 2005
Rainwater Mississippi	2007	Glyphosate: Median: 0.2 (<0.1–1.9) AMPA: Median: 0.1 (<0. 1–0.3)	Glyphosate and AMPA detected in 8/11 and 8/11 samples, respectively	Chang et al. 2011
	2008	Glyphosate: Median: 0.15 (<0.1–1.6) AMPA: Median: <0.1 (<0. 1–0.48)	Glyphosate and AMPA detected in 13/11 and 14/19 samples, respectively	
Rainwater Iowa	2007	Glyphosate: Median: 0.2 (<0.1–2.5) AMPA: Median: <0.1 (<0. 1–0.2)	Glyphosate and AMPA detected in 10/14 and 5/14 samples, respectively	Chang et al. 2011
	2008	Glyphosate: Median: 0.1 (<0.1–1.8) AMPA: Median: <0.1 (<0. 1–0.24)	Glyphosate and AMPA detected in 15/24 and 12/24 samples, respectively	
Rainwater Indiana	2004	Glyphosate: Median: 0.14 (<0.1–1.1) AMPA: Median: <0.1 (<0.1–47)	Glyphosate and AMPA detected in 11/12 and 11/12 samples, respectively	Chang et al. 2011

Table 5-8. Surface Water Monitoring Data for Glyphosate

Location	Date	Concentration (range) in µg/L	Notes	Reference
Rainwater Flanders, Belgium	2001	Maximum during spraying season: Glyphosate: 6,200 ng/L AMPA: 1,200 ng/L Average annual concentrations: Glyphosate: 78 ng/L AMPA: 20 ng/L	Glyphosate detected in 10% of samples; AMPA detected in 13% of samples	Quaghebeur et al. 2004

AMPA = aminomethylphosphonic acid; EPA = U.S. Environmental Protection Agency; MDL = method detection limit; STORET = STOrage and RETrieval; USGS = U.S. Geological Survey

Table 5-9. Groundwater Monitoring Data for Glyphosate

Location	Date	Concentration (µg/L)	Notes	Reference
Groundwater Wyoming	September 9, 2010	1.6	EPA STORET data: Routine monitoring sample from USGS Wyoming Water Science Center	WQP 2017
Groundwater Florida	March 2, 2010	0.14	EPA STORET data: Routine monitoring sample from USGS Florida Water Science Center	WQP 2017
Groundwater Louisiana	April, October, and November 2011	0.03–2.2	EPA STORET data: Routine monitoring sample from USGS Louisiana Water Science Center; depths 43.5–82 feet	WQP 2017
Groundwater Alabama Texas	February and April, 2012	0.01–0.06	EPA STORET data: Routine monitoring sample from USGS Alabama Water Science Center; USGS Texas Water Science Center	WQP 2017
Groundwater Kansas	June and August 2014, June 2015, July 2016	0.02–0.24	EPA STORET data: Routine monitoring sample from USGS Kansas Water Science Center	WQP 2017
Groundwater 23 U.S. states	2001–2010	Median: <0.02 Maximum: 2.03	Detected in 68 out of 1,171 samples	Battaglin et al. 2014

Table 5-9. Groundwater Monitoring Data for Glyphosate

Location	Date	Concentration (µg/L)	Notes	Reference
Groundwater Washington, DC	2008	0.02	Detected in 1 out of 13 well; not detected in 14 wells sampled in 2005	USGS 2010
Well water Minnesota	October and November 2014, 2015	Not detected	EPA STORET data: Routine monitoring sample from Minnesota Department of Agriculture Pesticide Monitoring Program; activity depth reported at 0 m	WQP 2017

EPA = U.S. Environmental Protection Agency; STORET = STOrage and RETrieval; USGS = U.S. Geological Survey

Table 5-10. Sediment and Soil Monitoring Data for Glyphosate

Location	Date	Concentration (µg/g)	Notes	Reference
Sediment Big Valley Rancheria, California	July 6, 2010	Not detected	EPA STORET data: Routine monitoring samples from Big Valley Band of Pomo Indians of the Big Valley Rancheria, California: two samples; depth: 0.152 m; MDL: 0.017 mg/kg	WQP 2017
Soil and sediment Indiana, Mississippi	2001–2010	Median: 0.0096; maximum: 0.476	Detected in >90% of 45 samples	Battaglin et al. 2014
Estuary Willapa Bay, Washington	July 1997–1999	1997 mudflat samples: 2.58–16.3 1998 mudflat samples: 3.11–9.94 1999 mudflat samples: 0.311–1.21 1997 meadow samples: 0.090–0.265 1998 meadow samples: 0.163–2.30 1999 meadow samples 0.472–1.32 (dry weight)	Aqueous herbicide formulated with Rodeo (5% solution v/v) and LI-700 (2% solution) applied in mudflat and cordgrass plots of land in 1997 and 1998	Kilbride and Paveglio 2001
Major river basins in the United States	2011–2006	193 samples collected; 119 glyphosate detections (0.001–0.476); 154 detections AMPA, (0.001–0.956)	Samples collected as part of USGS study	USGS 2007

AMPA = aminomethylphosphonic acid; EPA = U.S. Environmental Protection Agency; MDL = method detection limit; STORET = STOrage and RETrieval; USGS = U.S Geological Survey

Glyphosate concentrations found in edible food treated with formulations of Roundup® ranged from undetectable, ≤0.05 mg/kg, in several foods like bananas and selected meats to 3.7 mg/kg in a variety of grains and grain-based products (FAO 2005; FAO and WHO 2016). Genetically modified, and conventional food samples were studied. Herbicidal application techniques used on the food samples examined included pre-harvest application, directed ground spray, pre-emergence, and recirculating spray application methods. Application rates ranged from 0.36 to 7.7 kg/ha. The highest concentration found in banana pulp was 0.16 mg/kg. All kiwifruit assessed in the study had undetectable residues. Olives had residues ranging from undetectable to 12 mg/kg. Dry beans had residues ranging from undetectable to 10 mg/kg. Dry peas had residues ranging from undetectable to 8.9 mg/kg. Lentils had residues ranging from undetectable to 17 mg/kg. Glyphosate-tolerant sugar beet root had residues ranging from undetectable to 8.6 mg/kg. Conventional maize had residues ranging from undetectable to 3 mg/kg. Glyphosate-tolerant maize had residues ranging from undetectable to 0.83 mg/kg. Oats had residues ranging from undetectable to 19 mg/kg. Rye grain had residues ranging from 0.1 to 4.6 mg/kg. Wheat grain had residues ranging from 0.09 to 6.4 mg/kg. Sugarcane had residues ranging from undetectable to 15 mg/kg. Coffee and tea had levels ranging from undetectable to 9.6 mg/kg. Glyphosate residues in Kona Hawaiian coffee beans prior to roasting were 0.58 mg/kg, and the roasted beans had residues of 0.06 mg/kg.

Glyphosate was not included in compounds tested for by the Food and Drug Administration's (FDA) Pesticide Residue Monitoring Program (PRMP), nor in the United States Department of Agriculture's Pesticide Data Program (PDP) (FDA 2015; NPIC 2015).

A review by WHO reported that glyphosate was not detected in cereal grains at harvest when application of the herbicide occurred before planting (WHO 2005). Glyphosate was detected in cereals at mean residue levels of 0.2–4.8 mg/kg when application of the herbicide was prior to harvesting. In one assessment, levels of glyphosate were found to decrease upon industrial processing grains to flour from 1.6 to 0.16 mg/kg (WHO 2005). In wheat treated with either Glyphos or Roundup® herbicides, levels of glyphosate were also found to decrease upon processing grains to flour from 0.28–1.0 mg/kg in the grains to <0.05 mg/kg in the flour (FAO 2005). Glyphosate residues in oats stored at room temperature compared to frozen storage were similar, 3.5 and 3.1 mg/kg, respectively (FAO 2005). After exposure to glyphosate at 10 mg/L for 14 days, fish concentrations ranged from 0.2 to 0.7 mg/kg and decreased upon exposure to glyphosate-free water (WHO 2005).

A review by Williams et al. (2000) reported U.S. glyphosate residue data for wheat treated with maximum rates of Roundup®. Wheat crop residues consisted of a mean glyphosate concentration of 0.69 µg/g (mg/kg), with a maximum concentration of 2.95 µg/g (mg/kg). Glyphosate-tolerant soybeans treated with maximum rates of Roundup® showed a mean glyphosate concentration of 2.36 µg/g (mg/kg) and a maximum concentration of 5.47 µg/g (mg/kg).

Glyphosate was detected in carrot samples at average concentrations of 0.078±0.002 mg/kg and in spinach at 0.104±0.005 mg/kg (Zhao et al. 2011).

Glyphosate residues were examined on alder and salmonberry foliage and leaf litter sprayed with glyphosate at 2.0–2.1 kg/ha (Feng et al. 1990b). Foliar residues on alder and salmonberry were 261 and 448 ppm (dry weight), respectively, after the initial application of the herbicide. Leaf litter of alder and salmonberry collected 15 days post-application had glyphosate residues of 12.5 and 19.2 ppm (mg/kg), respectively. After 8–9 days, 50% dissipation was reported for the glyphosate residue. AMPA residues in the leaf litter decreased, and at 29 days after application of the herbicide, concentrations of AMPA were not detected.

5.6 GENERAL POPULATION EXPOSURE

The main routes of exposure to glyphosate for the general public result from the ingestion of foods with residues of glyphosate and foods made from these crops, as well as dermal, ocular, or inhalation exposure from application of herbicides containing glyphosate (EPA 2009c). Glyphosate has been detected in dust samples from homes near glyphosate application sites or from people who brought it indoors on their bodies and/or clothing from glyphosate-treated areas (Curwin et al. 2005). Upon dermal exposure, absorption through the skin is expected to be low based on dermal absorption studies, where an estimated 0.8–2.2% percutaneous absorption of glyphosate occurred in a study using ^{14}C-radiolabeled glyphosate in Roundup® (Wester et al. 1991). Evidence has shown that proper hygiene removes glyphosate from skin and will deter absorption through the skin (Wester et al. 1991). Limited monitoring data indicate that oral exposure may occur from drinking contaminated well water supplied from groundwater contaminated with glyphosate; concentrations reported in groundwater are relatively low, and this chemical has low leaching potential from soil to groundwater. Exposure may also occur via ingestion of food with herbicidal residues containing glyphosate as a result of its application. The FDA has not performed a total diet study on glyphosate. Glyphosate has not been included in the FDAs Pesticide Residue Monitoring Program Reports for the fiscal years 2009 through 2015 (FDA 2013a, 2013b, 2014, 2015,

2016, 2017); however, the FDA in 2016 and 2017 began preliminary testing of samples of soybeans, corn, milk, and eggs for glyphosate residues (FDA 2018). Preliminary results showed no pesticide residue violations for glyphosate in all four commodities tested (soybeans, corn, milk, and eggs). The Joint FAO/WHO Meeting on Pesticide Residues listed International Estimated Daily Intake (IEDI) of glyphosate from 17 GEMS/Food (Global Environment Monitoring System-Food Contamination Monitoring and Assessment Programme) cluster diets to range from 140.5 to 443.0 µg/person (FAO and WHO 2016). Glyphosate is a non-volatile compound, and drift of herbicidal sprays may occur with aerial and ground equipment (Yates et al. 1978); therefore, some exposure via inhalation and direct contact with skin and eyes may occur after members of the general population apply glyphosate during residential use. Glyphosate exposure of populations living in areas where glyphosate-containing products have been aerially-applied to eradicate coca crops has been evaluated (Paz-y-Miño et al. 2007, 2011; Solomon et al. 2009). For example, Paz-y-Miño et al. (2007) reported increased prevalence of DNA strand breaks in blood samples from 24 residents of an area in northern Ecuador following aerial applications of Roundup-Ultra®. Such reports did not include monitoring of exposure levels.

Occupational exposure may occur in both forestry, landscaping, and agricultural settings from the direct use of herbicides containing glyphosate. The most probable routes for occupational exposure are via inhalation and dermal contact with this chemical at workplaces where glyphosate or products containing this chemical are produced or used. Oral exposure may occur from accidental ingestion. During the years 1990–1993, exposure to glyphosate of field workers applying mixtures of Roundup® plus an additional herbicide in areas of Louisiana was assessed (LaDOTD 1995). Mixtures of Roundup® (active ingredient glyphosate) plus Garlon-3A (active ingredient triclopyr) and Roundup® (active ingredient glyphosate) plus 2,4-D (active ingredient 2,4-dichlorophenoxyacetic acid) were applied by 13 workers using spray equipment with operating capabilities of 0.37 L/minute. Glyphosate was detected in the workers urine using HPLC with a detection limit of 100 ppb. Total excreted urinary amounts ranging from non-detectable to 175 µg/day were reported for both working and non-working days. Urine concentrations were higher than concentrations found in the collected air samples of the breathing zone. It was noted that inhalation exposure was very low compared with threshold limits; the maximum air concentration was 17.9 µg/m^3. Dermal contact and improper hygiene leading to ingestion of the herbicides were noted as the probable routes of exposure.

Farmers, with an average age of 45 years licensed as pesticide applicators in South Carolina and Minnesota, who applied herbicides containing glyphosate had average urinary glyphosate levels of 3 µg/L on the day of application (Acquavella et al. 2004). Lack of wearing rubber gloves was associated with

higher concentrations in farmers' urine. Spouses, with an average age of 42.2 years residing with the farmers but having minimal or no involvement in the preparation or application of the herbicide, had relatively low and consistent urine concentrations, while children (ages 4–18 years) had an increase followed by a decrease in urine concentrations correlated with application (see Table 5-11). For the entire assessment period, 88–95% of all samples of children's urine were below the detection limit (1 µg/L [ppb] for a 100-mL urine sample). Farmers applying the pesticide had the highest concentrations. The highest concentration of glyphosate found in a child was from a teenage male (29 µg/L [ppb]) who had assisted with mixing and application of the herbicide. An estimated dermal and inhalation exposure value of about 8,000 µg/hour was reported as the highest value from a study of workers employing spray applicators; when corrected for incomplete absorption, this corresponds to an approximate exposure of 50 µg/kg body weight/day (8-hour working day for a 70-kg adult) (IPCS 1994).

Table 5-11. Human Monitoring Data

Medium		Concentrations/ minimum, maximum	Average	Notes	Reference
Tissue (brain, blood, liver, kidney)	Postmortem, approximately 12–13 hours after ingestion	Glyphosate (ppm): kidney 3,650; liver 600; blood; 550; brain; 100		After one individual ingested 200–250 mL Roundup® with 72–91 g/mL glyphosate	Menkes et al. 1991
Urine	Pre-application	<1–15 µg/L (ppb)	Not reported	Farmers applying pesticide; average age: 45 years	Acquavella et al. 2004
	Day of pesticide application	<1–233 µg/L (ppb)	Geometric mean: 3.2 µg/L (ppb)		
	1-Day post-pesticide application	<1–126 µg/L (ppb)	Geometric Mean: 1.7 µg/L (ppb)		
	2-Day post-pesticide application	<1–81 µg/L (ppb)	Geometric mean: 1.1 µg/L (ppb)		
	3-Day post-pesticide application	<1–68 µg/L (ppb)	Geometric mean: 1.0 µg/L (ppb)		
	Pre-application	<1–3 µg/L (ppb)	Not reported	Spouses not involved with application; average age: 42 years	
	Day of pesticide application	<1–2 µg/L (ppb)	Not reported		
	1–3-Day post-pesticide application	<1–1 µg/L (ppb)	Not reported		

Table 5-11. Human Monitoring Data

Medium		Concentrations/ minimum, maximum	Average	Notes	Reference
	Pre-application	<1–17 µg/L (ppb)	Not reported	Children not involved with application; average age: 11.5 years	
	Day of pesticide application	<1–29 µg/L (ppb)	Not reported		
	1-Day post-pesticide application	<1–24 µg/L (ppb)	Not reported		
	2-Day post-pesticide application	<1–12 µg/L (ppb)	Not reported		
	3-Day post-pesticide application	<1–6 µg/L (ppb)	Not reported		
	Daily during 1-week working period	<0.1 ng/µL		Forest workers using pressurized herbicide sprayers; 8% Roundup® (active ingredient 360 g/L isoproylamine salt)	Jauhiainen et al. 1991
	3 Weeks after 1-week working period	<0.1 ng/µL			
	Following mild to fatal ingestions of 20–500 mL pesticide	Glyphosate: 228 mg/L mild/moderate case; 22,300 mg/L fatal case; AMPA: 0.54 mg/L mild/moderate case; 91.5 mg/L fatal case		13 individuals ages 25–69 years	Zouaoui et al. 2013
	Two occasions (1 month apart) during spring and summer of 2001 (LOD 0.9 µg/L)	0.13–5.4 µg/L	1.4 µg/L	Farm fathers	Curwin et al. 2007b
		0.20–18 µg/L	1.9 µg/L	Non-farm fathers	
		0.062–5.0 µg/L	1.2 µg/L	Farm mothers	
		0.10–11 µg/L	1.5 µg/L	Nonfarm mothers	
		0.10–9.4 µg/L	2.7 µg/L	Farm children	
		0.022–18 µg/L	2 µg/L	Non-farm children	
Blood	Following mild to fatal ingestions of 20–500 mL pesticide	Glyphosate: 3.7 mg/L mild/moderate case; 6,640 mg/L fatal case; AMPA: 0.13 mg/L mild/moderate case; 15.4 mg/L fatal case			Zouaoui et al. 2013

AMPA = aminomethylphosphonic acid; LOD = limit of detection

Acquavella et al. (1999) evaluated 1,513 reported cases to the American Association of Poison Control Centers during the years 1993–1997 of ocular or dermal/ocular exposure to Roundup® herbicides with glyphosate concentrations ranging from <2 to >20%. Of all exposure cases, 62% involved male subjects, >80% were in a residential setting, and about 15% were in occupational settings. During the time period, California and Texas had the greatest number of reported cases. Dilute Roundup® formulations accounted for about 82% of the exposures; 5% were with concentrated Roundup®.

Aris and LeBlanc (2011) examined blood concentrations of glyphosate in a group of 30 pregnant and 39 non-pregnant females residing in Sherbrooke, Canada. The study noted that none of the subjects worked or lived with an individual who worked with pesticides. Neither glyphosate nor AMPA were detected in the maternal or fetal cord serum of pregnant subjects. Additionally, AMPA was not detected in non-pregnant subjects. Glyphosate was detected in 5% of the non-pregnant subjects at a range of not detectable to 93.6 ng/mL, with a mean of 73.6 ng/mL (LOD=15 ng/mL).

The Fourth National Report on Human Exposures to Environmental Chemicals, published and updated by the Centers for Disease Control and Prevention reporting biomonitoring data from the National Health and Nutrition Examination Survey (NHANES), does not include data for glyphosate or its metabolite, AMPA (CDC 2018).

As with the adult general population, exposure of children to glyphosate may occur through ingestion of foods with residues of glyphosate and foods made from these crops, as well as inhalation, dermal contact, and/or ocular contact when in the proximity of areas where glyphosate containing herbicides have been recently applied. Glyphosate has been detected in dust samples from homes near glyphosate application sites or from people who brought it indoors on their bodies and/or clothing from glyphosate-treated areas (Curwin et al. 2005). Limited monitoring data indicate that oral exposure may occur from drinking contaminated well water supplied from groundwater contaminated with glyphosate; concentrations reported in groundwater are relatively low, and this chemical has low leaching potential from soil to groundwater. Glyphosate is not likely to bioaccumulate in breast milk (Bus 2015) and was not detected in breast milk from lactating mothers with detectable glyphosate in their urine (McGuire et al. 2016); therefore, a determination of the importance of this route of child exposure has not been made.

During the spring and summer of 2001, urinary pesticide concentrations were investigated in families residing in non-farm and farm households located in Iowa (Curwin et al. 2007a, 2007b). Urinary glyphosate levels were fairly similar between farm and non-farm households. In addition, glyphosate concentrations were fairly similar when comparing individuals living on farms where the pesticide was used with those living on farms where the pesticide was not used. Glyphosate was detected at urinary levels equal to or greater than the LOD (0.9 µg/L) in 66% of the 23 non-farm fathers, 75% of the 24 farm fathers, 65% of the 24 non-farm mothers, 67% of the farm mothers, 88% of the non-farm children, and 81% of the farm children (Curwin et al. 2007b). Estimated glyphosate intakes among 40 children (17 homes) living on farms where glyphosate was applied ranged from 0.001 to 0.33 µg/kg/day, with 16%

of the samples below the LOD (Curwin et al. 2007a). Estimated glyphosate intakes among 25 children (8 homes) living on farms where glyphosate was not applied ranged from 0.003 to 0.64 µg/kg/day, with 20% of the samples below the LOD.

McQueen et al. (2012) estimated the mean glyphosate dietary exposure of 43 pregnant women at 0.001 mg/kg body weight/day and these exposures were well below applicable health guidelines. Since only a small percentage of glyphosate crosses the placenta, fetal exposure resulting from maternal exposure to glyphosate was minimal.

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

Farm workers, farming families, landscaping workers, and people of all ages living and or working in agricultural sectors will incur higher exposure to glyphosate, as agriculture is the largest industry for herbicide use. Field workers who apply herbicides containing glyphosate will likely incur higher exposures to this chemical. Levels of glyphosate in field workers urine has been shown to increase during spraying season; however, glyphosate levels did not appear to carry over from previous seasons (LaDOTD 1995).

CHAPTER 6. ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of glyphosate is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research designed to determine the adverse health effects (and techniques for developing methods to determine such health effects) of glyphosate.

Data needs are defined as substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

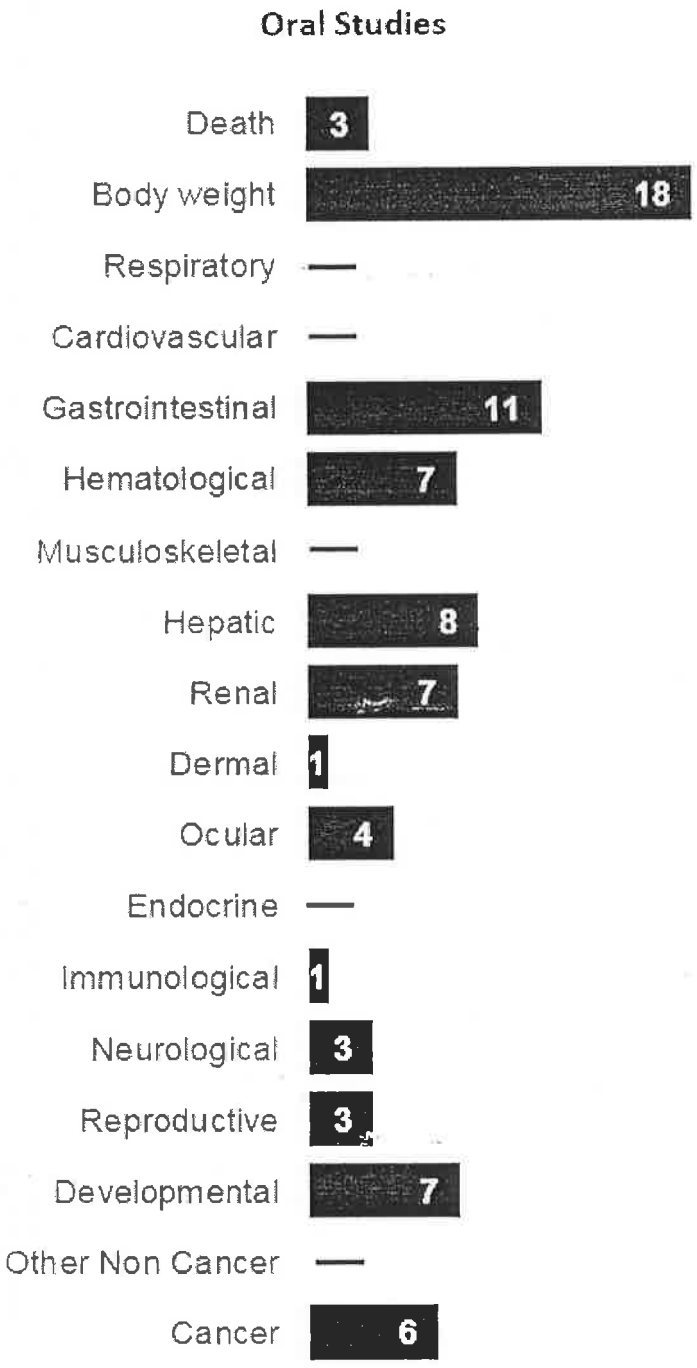
6.1 Information on Health Effects

Studies evaluating the health effects of inhalation, oral, and dermal exposure of humans and animals to glyphosate that are discussed in Chapter 2 are summarized in Figure 6-1 for glyphosate technical and Figure 6-2 for glyphosate formulations. The purpose of these figures is to illustrate the information concerning the health effects of glyphosate. The number of human and animal studies examining each endpoint is indicated regardless of whether an effect was found and the quality of the study or studies.

The health effects of glyphosate have been evaluated in epidemiology and animal studies. Epidemiological studies are predominantly case-control and cohort epidemiology studies that examined possible associations between glyphosate exposure and selected health outcomes (noncancer and cancer endpoints), or case reports following accidental or intentional ingestion of glyphosate-containing products. These studies do not include data regarding the extent of the exposure or relative contribution of inhalation, oral and/or dermal exposure. Most health effects data come from animal studies that employed oral exposure and examined potential body weight, gastrointestinal, hematological, hepatic, and/or developmental effects.

Figure 6-1. Summary of Existing Health Effects Studies of Animals Orally Exposed to Glyphosate Technical (Listed by Endpoint)*

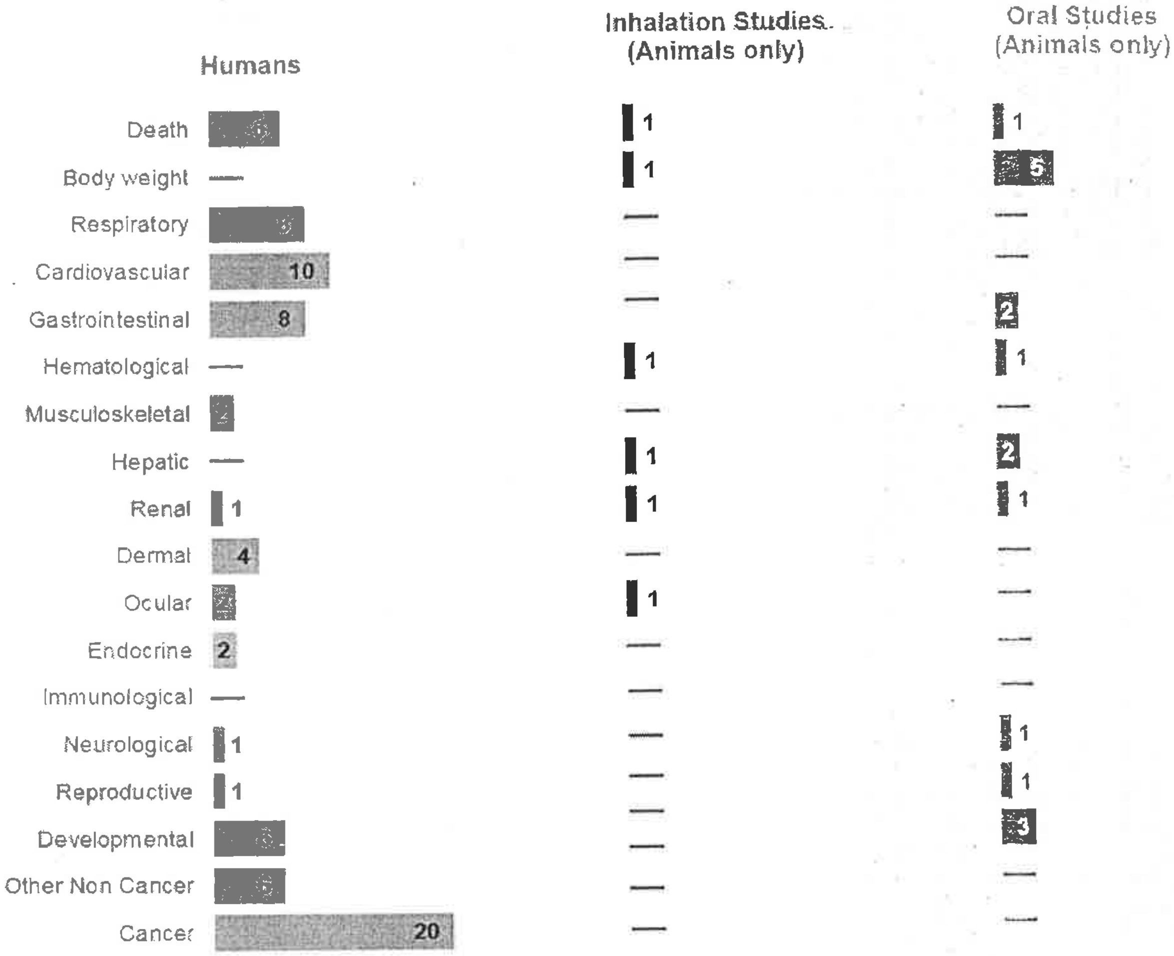
Potential body weight and gastrointestinal effects of glyphosate technical were the most studied endpoints



*Includes studies discussed in Chapter 2; the numbers of studies include those finding no effect.

Figure 6-2. Summary of Existing Health Effects Studies on Glyphosate Formulations (Listed by Endpoint)*

Potential cancer, respiratory, and developmental effects were the most studied in humans; potential body weight and developmental effects were the most studied in animals



*Includes studies discussed in Chapter 2; the numbers of studies include those finding no effect. Human exposures likely included multiple exposure routes.

6.2 Identification of Data Needs

Missing information in Figures 6-1 and 6-2 should not be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

Oral studies in animals indicate that glyphosate technical toxicity is expressed only at oral dose levels many times higher than levels allowed as residues in food products. The general population is most likely to be exposed to glyphosate residues in food sources. Humans should continue to be monitored for possible associations between glyphosate intake from food sources and adverse health outcomes. Individuals can also be exposed to glyphosate via inhalation, dermal contact, and/or ocular contact during application of the herbicide or by being in the vicinity where it is applied. However, available dermal studies indicate that only 3–4% of dermally-applied glyphosate enters the blood. Data regarding the extent of absorption and potential health effects following inhalation exposure are lacking. Therefore, human and animal studies should be designed to evaluate airborne exposure levels and possible health effects from inhalation exposure. Additional animal studies should be designed to assess the toxic effects of exposure to a variety of glyphosate formulations and individual components suspected to be toxic. Such studies could also be designed to evaluate possible interactions among individual components that might enhance toxicity.

Acute-, Intermediate-, and Chronic-Duration MRLs. No inhalation MRLs were derived for glyphosate due to the lack of quantitative exposure-response data for humans or animals.

As stated previously, most information is available from animal studies submitted to EPA's Office of Pesticides Programs using glyphosate technical (typically >90% purity) to fulfill requirements for the registration of a particular glyphosate formulation for use in the United States. Some animal studies in the open literature used glyphosate formulations that typically included 1–41% glyphosate technical (or glyphosate salts) and up to 18% surfactant (along with other "inert" ingredients). Surfactants in glyphosate formulations may be at least partly responsible for the toxic effects from overexposure to glyphosate formulations (Adam et al. 1997; Sawada et al. 1988; Williams et al. 2000). Human exposure to glyphosate formulations via its use in weed control includes exposure to all substances in a particular glyphosate formulation as well as to other substances that may be added by the end user. No MRLs were

derived for glyphosate formulations due to the wide variation in glyphosate content and surfactants used in various glyphosate formulations and the fact that surfactants can contribute to the toxicity of glyphosate formulations. However, because exposures of the general population via food or water sources with measurable glyphosate residues most likely involve glyphosate and/or its breakdown products rather than the intact glyphosate-based formulation, health effects data associated with oral exposure to glyphosate technical are considered relevant to potential derivation of oral MRLs for glyphosate. Oral MRLs based on glyphosate technical would not be applicable to intentional or accidental ingestion of a glyphosate formulation.

Provisional acute- and chronic-duration oral MRLs were derived for glyphosate based on gastrointestinal effects in animal studies. The provisional chronic-duration oral MRL was adopted as the provisional intermediate-duration oral MRL.

Health Effects

Respiratory. Limited information was located regarding the effects of inhalation exposure in laboratory animals. A single 4-week repeated-exposure rat study found no effects at the highest exposure concentration tested (36 mg Roundup®/m^3). Studies should be designed to evaluate respiratory effects in animals exposed to glyphosate by inhalation.

Developmental. Developmental toxicity studies in animals that employed oral exposure to glyphosate technical found no evidence of treatment-related effects at levels below the threshold of maternal toxicity. One study reported testicular lesions in weanling rats administered a glyphosate formulation orally at doses as little as 5 mg/kg/day. Additional studies should be designed to substantiate or refute this finding and to determine whether glyphosate or other ingredients in glyphosate formulations are involved in developmental effects on male reproductive organs.

Epidemiology and Human Dosimetry Studies. Limited information was located regarding respiratory effects associated with human exposure to glyphosate-based formulations. Additional studies should be designed to monitor exposure levels and health effects associated with individuals involved in the application of glyphosate-based products. There is limited evidence for glyphosate-related developmental effects in humans. Additional studies should be designed to evaluate possible associations between exposure to glyphosate and developmental endpoints in humans. Numerous agencies have evaluated glyphosate for possible associations between exposure and risk of various cancers. The

majority of the human studies used self-reported ever/never glyphosate use as the biomarker of exposure. The results of these studies should be interpreted cautiously given the lack of quantitative or semi-quantitative glyphosate exposure information and the likely exposure to other pesticides. Most studies found no association between exposure to glyphosate-based products and risk of cancer. However, a possible association between exposure to glyphosate and risk of non-Hodgkin's lymphoma could not be ruled out, based on conflicting results.

Biomarkers of Exposure and Effect. The most reliable biomarker of exposure to glyphosate is its detection in blood and urine. It is not likely that additional biomarkers of exposure to glyphosate would be more effective.

Absorption, Distribution, Metabolism, and Excretion. The toxicokinetics of glyphosate following oral and dermal exposure have been adequately described. Additional studies should be designed to evaluate the toxicokinetics of inhaled glyphosate.

Comparative Toxicokinetics. Significant species differences in the toxicokinetics of glyphosate are not likely.

Children's Susceptibility. Age-related differences in susceptibility to glyphosate have not been elucidated. Due to relatively large oral doses required to elicit adverse effects in glyphosate-exposed animals, it may be difficult to evaluate age-related differences in susceptibility. As additional epidemiological data become available, age-related issues regarding susceptibility to glyphosate toxicity should be evaluated.

Physical and Chemical Properties. The physical chemical properties of glyphosate are summarized in Chapter 4. No data needs are identified.

Production, Import/Export, Use, Release, and Disposal. No information is available in the TRI database on facilities that manufacture or process glyphosate because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 2005b). There is no information on releases of glyphosate from manufacturing and processing facilities because these releases are not required to be reported (EPA 2005b). Data on current manufacturing, processing, import/export values

would be useful information. Data on current uses and disposal practices are outlined in Sections 5.2.3 and 5.2.4. Further studies on these practices do not appear to be essential.

Environmental Fate. Transport, partitioning, and bioconcentration data are available for glyphosate summarized in Section 5.4. In glyphosate-tolerant plants, glyphosate is converted to N-acetylglyphosate; therefore, studies evaluating the possibility of additional crop and plant metabolites, along with the characteristic fates, may be beneficial (Pioneer 2006). Additional studies should be designed to further assess potential for glyphosate to persist in foods, water, and soil.

Bioavailability from Environmental Media. Glyphosate degrades quickly in the environment and adsorbs to soils and sediment and possesses low bioconcentration in aquatic organisms, suggesting that bioavailability from environmental media is low. A study regarding the bioavailability of glyphosate in soil indicated that degradation rates decreased in lower soil horizons as microbial populations of glyphosate degrading organisms decreased, but bioremediation practices that incorporate anthropic bacteria can be useful to remediate highly polluted glyphosate-containing soils and maintain low bioavailability (Shushkova et al. 2010). Additional studies on glyphosates bioavailability from different types of soil would be helpful to expand our understanding of potential human exposures to glyphosate bound residues.

Food Chain Bioaccumulation. Studies are available that indicate that glyphosate has very low potential to bioconcentrate in aquatic organisms and is not expected to bioaccumulate in the food chain. No data needs are identified.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of glyphosate in environmental media surrounding areas where it is applied are available (Chang et al. 2011; USGS 2007; WQP 2017). The USGS NAWQA frequently reports on levels of glyphosate and other substances in both surface water and groundwater. No data needs are identified; however, continued monitoring studies in air, water, soil, and other environmental media should continue as this is an herbicide used globally.

Exposure Levels in Humans. Studies are needed to investigate human intake of glyphosate via food and water, such as total diet studies. Up until 2016–2017, the FDA did not test for glyphosate residues in food sources because its multi-residue testing protocols did not include glyphosate. The FDA has now developed a method to specifically test for glyphosate residues in foods and results are expected

to be provided through the FDA Pesticide Residue Monitoring Program (FDA 2018). Biomonitoring information of glyphosate for the general population is needed.

Exposures of Children. Monitoring of children's exposure to glyphosate would be useful, in combination with children's health and susceptibility information, to assess the potential risk for deleterious effects.

Analytical Methods. Standardized methods that yield low detection limits for glyphosate and AMPA in biological samples (e.g., urine analysis, blood analysis) may provide more sensitivity and a more complete exposure analysis.

6.3 Ongoing Studies

Glyphosate is a potential candidate for addition to the California Environmental Contaminant Biomonitoring Program (CDPH 2013). Ongoing research identified in the National Institutes of Health (NIH) RePORTER (2017) database is summarized in Table 6-1. In addition, NTP (2017) is performing research to investigate potential genetic and mechanistic toxicity of glyphosate and glyphosate formulations. NTP will also evaluate published literature for information regarding glyphosate on non-cancer outcomes. Researchers at the Cesare Maltoni Cancer Research Centre at the Ramazzini Institute in Italy are conducting research into potential genetic, reproductive, and developmental effects in rats administered glyphosate at levels equivalent to those allowed in humans.

Table 6-1. Ongoing Studies on Glyphosate

Investigator	Affiliation	Research description	Sponsor
De Roos, AJ	Drexel University	Occupational pesticide use and risk of lymphoid cancers	National Cancer Institute
Keating, AF	Iowa State University	Investigating modes of action of glyphosate-induced ovotoxicity	National Institute of Environmental Health Sciences

Source: RePORTER 2017

CHAPTER 7. REGULATIONS AND GUIDELINES

Pertinent international and national regulations, advisories, and guidelines regarding glyphosate in air, water, and other media are summarized in Table 7-1. This table is not an exhaustive list, and current regulations should be verified by the appropriate regulatory agency.

ATSDR develops MRLs, which are substance-specific guidelines intended to serve as screening levels by ATSDR health assessors and other responders to identify contaminants and potential health effects that may be of concern at hazardous waste sites. See Section 1.3 and Appendix A for detailed information on the provisional MRLs for glyphosate.

Table 7-1. Regulations and Guidelines Applicable to Glyphosate

Agency	Description	Information	Reference
	Air		
EPA	RfC	Not evaluated	IRIS 1989
WHO	Air quality guidelines	No data	WHO 2010
	Water & Food		
EPA	Drinking water standards and health advisories		EPA 2012d
	1-Day (10-kg child)	20 mg/L	
	10-Day (10-kg child)	20 mg/L	
	DWEL	70 mg/L	
	RfD	2.0 mg/kg/day[a]	
	National primary drinking water regulations		EPA 2009b
	Maximum Contaminant Level	0.7 mg/L	
	Public Health Goal	0.7 mg/L	
	RfD	0.1 mg/kg/day[b]	IRIS 1989
WHO	Drinking water quality guidelines	Not established[c]	WHO 2017
FDA	EAFUS	No data[d]	FDA 2013c
	Cancer		
HHS	Carcinogenicity classification	No data	NTP 2016
EPA	Carcinogenicity classification	Group D[e]	IRIS 1989
IARC	Carcinogenicity classification	Group 2A[f]	IARC 2017
	Occupational		
ACGIH	TLV	No data	ACGIH 2016
OSHA	PEL (8-hour TWA) for general industry	No data	OSHA 2016b
	PEL (8-hour TWA) for shipyards and construction	No data	OSHA 2016c
	PEL (8-hour TWA) for construction	No data	OSHA 2016a
NIOSH	REL (up to 10-hour TWA)	No data	NIOSH 2016

Table 7-1. Regulations and Guidelines Applicable to Glyphosate

Agency	Description	Information	Reference
	Emergency Criteria		
EPA	AEGLs-air	No data	EPA 2016b
DOE	PACs-air	No data	DOE 2018

[a]EPA's Office of Pesticides Program (OPP) is presently re-evaluating glyphosate in its Registration Review program.
[b]EPA's IRIS program has not planned to re-evaluate the RfD for glyphosate, which was based on increased incidence of renal tubular dilation in F3b offspring of rats receiving glyphosate from the diet at 30 mg/kg/day (EPA 1992g).
[c]Glyphosate and aminomethylphosphonic acid occur in drinking water at concentrations well below those of health concern, so a guideline value was not deemed necessary.
[d]The EAFUS list of substances contains ingredients added directly to food that FDA has either approved as food additives or listed or affirmed as GRAS.
[e]Group D not classifiable as to human carcinogenicity. Note: EPA's IRIS program has not planned to re-evaluate the potential carcinogenicity of glyphosate. EPA's Office of Pesticide Programs (EPA 2015c) re-evaluated available human and animal data regarding the potential carcinogenicity of glyphosate and concluded that the strongest support was for the descriptor "*not likely to be carcinogenic to humans* at doses relevant to human risk assessment."
[f]Group 2A: Probably carcinogenic to humans.

ACGIH = American Conference of Governmental Industrial Hygienists; AEGL = acute exposure guideline levels; CFR = Code of Federal Regulations; DOE = Department of Energy; DWEL = drinking water equivalent level; EAFUS = Everything Added to Food in the United States; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; HHS = Department of Health and Human Services; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; PAC = Protective Action Criteria; PEL = permissible exposure limit; REL = recommended exposure limit; RfC = inhalation reference concentration; RfD = oral reference dose; TLV = threshold limit values; TWA = time-weighted average; WHO = World Health Organization

CHAPTER 8. REFERENCES

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APPENDIX A. ATSDR MINIMAL RISK LEVEL WORKSHEETS

MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified route and duration of exposure. MRLs are based on noncancer health effects only; cancer effects are not considered. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the NOAEL/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (≥365 days) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive substance-induced endpoint considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as 100-fold below levels that have been shown to be nontoxic in laboratory animals.

APPENDIX A

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology and Human Health Sciences, expert panel peer reviews, and agency-wide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published MRLs. Newly-derived and revised MRLs are designated "provisional" MRLs prior to publication of the final post-public comment draft of each toxicological profile, at which time the "provisional" designation is removed. For additional information regarding MRLs, please contact the Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, Georgia 30329-4027.

Human exposure to glyphosate formulations via its use in weed control includes exposure to all substances in a particular glyphosate formulation. No MRLs were derived for glyphosate formulations due to the wide variation in glyphosate content and surfactants used in various glyphosate formulations and the fact that surfactants can contribute to the toxicity of glyphosate formulations. However, the general population may be exposed via food or water sources containing glyphosate residues from glyphosate-based formulations registered for use in agricultural and residential environments. Therefore, health effects data associated with oral exposure to glyphosate technical are considered relevant to potential derivation of oral MRLs for glyphosate.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name:	Glyphosate technical
CAS Numbers:	1071-83-6
Date:	April 2019
Profile Status:	Draft for Public Comment
Route:	Inhalation
Duration:	Acute

MRL Summary: There are insufficient data for derivation of an acute-duration inhalation MRL.

Rationale for Not Deriving an MRL: No acute-duration inhalation exposure-response studies were identified for glyphosate.

Agency Contact (Chemical Manager): Hana R. Pohl, M.D., Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name:	Glyphosate technical
CAS Numbers:	1071-83-6
Date:	April 2019
Profile Status:	Draft for Public Comment
Route:	Inhalation
Duration:	Intermediate

MRL Summary: There are insufficient data for derivation of an intermediate-duration inhalation MRL.

Rationale for Not Deriving an MRL: No intermediate-duration inhalation exposure-response studies were identified for glyphosate.

Agency Contact (Chemical Manager): Hana R. Pohl, M.D., Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name:	Glyphosate technical
CAS Numbers:	1071-83-6
Date:	April 2019
Profile Status:	Draft for Public Comment
Route:	Inhalation
Duration:	Chronic

MRL Summary: There are insufficient data for derivation of a chronic-duration inhalation MRL.

Rationale for Not Deriving an MRL: No chronic-duration inhalation exposure-response studies were identified for glyphosate.

Agency Contact (Chemical Manager): Hana R. Pohl, M.D., Ph.D.

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Glyphosate technical
CAS Numbers: 1071-83-6
Date: April 2019
Profile Status: Draft for Public Comment
Route: Oral
Duration: Acute
MRL: 1 mg/kg/day (provisional)
Critical Effect: Gastrointestinal effects
Reference: EPA 2017b
Point of Departure: NOAEL of 100 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 6
Species: Rabbit

MRL Summary: A provisional acute-duration oral MRL of 1 mg/kg/day was derived for glyphosate based on gastrointestinal effects (diarrhea, few feces) observed in pregnant female New Zealand white rabbits administered glyphosate acid (96.5% purity) by daily gavage (in deionized water) during GDs 8–20 EPA (2017b). The provisional MRL is based on a NOAEL of 100 mg/kg/day and a total uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

Selection of the Critical Effect: Several acute-duration oral studies were available regarding the toxicity of glyphosate technical following acute-duration oral exposure (see Table A-1). The lowest LOAELs were 175 mg/kg/day for gastrointestinal effects (diarrhea, few feces) in maternal rabbits and 300 mg/kg/day for developmental effects (depressed fetal weight) following gavage treatment with glyphosate technical during GDs 8–20 at 175 mg/kg/day. Based on available data, gastrointestinal disturbance is considered to represent the most sensitive effect of glyphosate toxicity following oral exposure in laboratory animals.

Table A-1. NOAELs and LOAELs Identified in Acute-Duration Oral Studies of Glyphosate Technical

Endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Body weight	28.5% depressed maternal body weight gain in rats	1,000	3,500	EPA 1992e
	No effect in pregnant rats	1,000		EPA 2017b
	No effect in pregnant rabbits	300		EPA 2017b
Gastrointestinal	Diarrhea in 2/8 rats gavaged once		2,000	Adam et al. 1997
	Diarrhea in rats gavaged once	1,000	2,000	EPA 2013c
	Diarrhea, soft stools in pregnant rats gavaged on GDs 6–19	1,000	3,500	EPA 1992e
	Diarrhea, few feces in pregnant rabbits gavaged on GDs 8–20	100	175	EPA 2017b

Table A-1. NOAELs and LOAELs Identified in Acute-Duration Oral Studies of Glyphosate Technical

Endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Developmental	Decreased fetal weight; delayed ossification	1,000	3,500	EPA 1992e
	No effect in fetuses from pregnant rats gavaged on GDs 7–16	1,000		EPA 2017b
	Depressed weight in fetuses from pregnant rabbits gavaged on GDs 8–20	175	300	EPA 2017b
Other	Hypothermia in rats gavaged once	1,000	2,000	EPA 2013c

GD = gestation day; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level

Selection of the Principal Study: Among available acute-duration oral toxicity studies for glyphosate, the developmental toxicity study in rabbits (EPA 2017b) identified the lowest LOAEL (gastrointestinal effects in pregnant rabbits gavaged with glyphosate acid); the corresponding NOAEL was 100 mg/kg/day. Therefore, this study was selected as the principal study for deriving a provisional acute-duration oral MRL for glyphosate.

Summary of the Principal Study:

EPA. 2017b. Memorandum. December 13, 2017. Glyphosate: Preparation of data evaluation records for developmental rat and rabbit toxicity studies. MRID No.: 43320615, 43320616. Washington, DC: U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention.

Groups of sperm-positive female New Zealand white rabbits (20/group) were administered glyphosate acid (95.6% active ingredient) by daily gavage (in deionized water vehicle; dosing volume 2 mL/kg body weight) on GDs 8–20 at target concentrations of 0, 100, 175, or 300 mg/kg/day (adjusted for purity of active ingredient). Dams were monitored for survival, clinical signs, body weight, and food intake. On GD 30, dams were sacrificed and subjected to gross external and internal examination, pregnancy status, weight of gravid uteri, number of corpora lutea, number and position of implantations, live fetuses, and early and late intrauterine deaths. Fetuses were evaluated for weight and sex. External, visceral, and skeletal examinations were performed; brains were subjected to macroscopic examination.

The 100 mg/kg/day dose level represented a NOAEL for maternal toxicity. At 175 and 300 mg/kg/day, maternal rabbits exhibited diarrhea and reduced production of feces. Mean body weight in the 300 mg/kg/day group of maternal rabbits ranged from 5.2 to 7.4% less than that of controls during GDs 16–26. The depressed maternal body weight was <10% in magnitude, and was therefore not considered to represent an adverse effect. Furthermore, there were no statistically significant differences between controls and glyphosate-treated groups regarding GD 30 mean maternal body weight. Gross pathologic examination of maternal rabbits revealed no treatment-related effects. There were no treatment-related effects on pregnancy rate, numbers of corpora lutea, total number of implantation sites, litter size, sex ratio, or pre- or post-implantation loss. The 300 mg/kg/day dose group exhibited 8.3% lower mean fetal weight ($p<0.05$). Gross and visceral examination of fetuses revealed no treatment-related effects. Increased incidences of fetuses with selected minor skeletal defects (e.g., delayed sternebral and vertebral ossification) were observed at the 300 mg/kg/day maternal dose level. However, incidences of these skeletal defects did not appear to be increased in glyphosate-treated groups when

evaluated on a per litter basis; therefore, they were not considered treatment-related developmental effects.

Selection of the Point of Departure: Incidence data for the gastrointestinal effects were not presented in the available data evaluation record (DER) for the study, thus precluding a benchmark dose (BMD) approach to deriving an MRL. Therefore, the NOAEL of 100 mg/kg/day was selected as the point of departure for deriving a provisional acute-duration oral MRL for glyphosate.

Uncertainty Factor: The NOAEL of 100 mg/kg/day was divided by a total uncertainty factor of 100:

- 10 for animal to human extrapolation
- 10 for human variability

Other Additional Studies or Pertinent Information: Glyphosate-induced gastrointestinal effects were observed in acute-duration oral studies of rats (Adam et al. 1997; EPA 1992e, 2013c), although rabbits appear to be much more sensitive than rats to glyphosate-induced gastrointestinal effects following oral dosing.

Agency Contacts (Chemical Managers): Hana R. Pohl, M.D., Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Glyphosate technical
CAS Numbers: 1071-83-6
Date: April 2019
Profile Status: Draft for Public Comment
Route: Oral
Duration: Intermediate

MRL Summary: The provisional chronic-duration oral MRL of 1 mg/kg/day is adopted as the provisional intermediate-duration oral MRL.

Rationale for Not Deriving an MRL: Several intermediate-duration oral animal studies were available for glyphosate technical (see Table A-2).

Table A-2. NOAELs and LOAELs Identified in Intermediate-Duration Oral Studies of Glyphosate Technical

Endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Body weight	12–18% depressed paternal body weight gain in rats	M: 754 F: 802	M: 2,219 F: 3,134	EPA 1992a
	No effect in rats (highest dose)	M, F: 30		EPA 1992g
	No effect in rats (highest dose)	M: 1,234 F: 1,273		EPA 2013a
	18% lower mean body weight and body weight gain in male rats	M: 1,678 F: 3,393	M: 3,393	NTP 1992
	No effect in mice (highest dose)	F: 1,447.5		EPA 2013b
	10–11% lower mean final body weight in mice	M: 2,273 F: 5,846	M: 4,776 F: 11,977	NTP 1992
	No effect in maternal rabbits (highest dose)	F: 350		EPA 1992f
Gastrointestinal	Soft stool in rats	M: 754 F: 802	M: 2,219 F: 3,134	EPA 1992a
	Increased severity of basophilia and hypertrophy of acinar cells in parotid and submandibular salivary glands of rats	M: 205 F: 213	M: 410 F: 421	NTP 1992
	Increased severity of basophilia of acinar cells in parotid salivary gland of mice	M: 1,065 F: 1,411	M: 2,273 F: 2,707	NTP 1992
	Increased incidence of soft stool and/or diarrhea in pregnant rabbits	175	350	EPA 1992f
Hematological	No effect in rats (highest dose)	M, F: 3,393		NTP 1992

Table A-2. NOAELs and LOAELs Identified in Intermediate-Duration Oral Studies of Glyphosate Technical

Endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Hepatic	No effect in rats (highest dose)	M: 1,234 F: 1,273		EPA 2013a
	M: Increases in liver weight and serum ALT F: Increases in liver weight and serum AP, ALT, and bile acids	M: 811 F: 1,690	M: 1,678 F: 3,393	NTP 1992
	No effect in mice	M: 10,780 F: 11,977		NTP 1992
Renal	No effect in rats (highest dose)	M: 1,234 F: 1,273		EPA 2013a
Immunological	No effect in mice (highest dose)	F: 1,447.5		EPA 2013b
Neurological	No effect in rats (highest dose)	M: 1,546.5 F: 1,630.6		EPA 2013c
Reproductive	No effect in rats (highest dose)	M: 2,219 F: 3,234		EPA 1992a
	No effect in rats (highest dose)	M, F: 30		EPA 1992g
	No effect in rats (highest dose)	M: 1,234 F: 1,273		EPA 2013a
Developmental	14–20% depressed pup body weight during lactation (maternally toxic dose level)	802	3,134	EPA 1992a
	Delayed preputial separation	408	1,234	EPA 2013a
	No effect in rabbits (highest dose)	350		EPA 1992f

ALT = alanine aminotransferase; AP = alkaline phosphatase; F = female; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level

Increased incidence of kidney tubular dilation was reported for F3b male weanlings of a 3-generation study of glyphosate technical (98.7% purity) administered to male and female Sprague-Dawley rats in the diet at an estimated dose level of 30 mg/kg/day; the reported NOAEL was 10 mg/kg/day (EPA 1992g). However, there were no signs of treatment-related effects on kidneys of rat offspring in two subsequent 2-generation rat studies at dietary doses up to 1,234 or 1,273 mg/kg/day for parental males and females, respectively (EPA 2013a), or 2,633 or 3,134 mg/kg/day for parental males and females, respectively (EPA 1992a). Therefore, the finding of increased incidence of kidney tubular dilation in the 3-generation rat study (EPA 1992g) was considered a spurious result rather than a glyphosate-induced adverse developmental effect. In one 2-generation oral rat study, exposure via the diet at estimated parental dose levels of 1,234 or 1,273 mg/kg/day (parental males and females, respectively) resulted in delayed preputial separation in male pups (EPA 2013a). In the other 2-generation study, the highest dietary dose level (up to 2,633 and 3,134 mg/kg/day for parental males and females, respectively) resulted in up to 14–20% depressed pup body weight and/or body weight gain during the lactation period (EPA 1992a). There were no apparent treatment-related developmental effects in a study of rabbits treated by gavage at up to 350 mg/kg/day during GDs 6–27 (EPA 1992f).

As shown in Table A-2, gastrointestinal endpoints are the most sensitive to intermediate-duration oral exposure of laboratory animals to glyphosate technical. Pregnant rabbits gavaged with glyphosate technical daily at 350 mg/kg/day (LOAEL) during GDs 6–27 exhibited increased incidence of soft stool and/or diarrhea; the NOAEL was 175 mg/kg/day (EPA 1992f). Similar results were observed among other pregnant rabbits gavaged daily with glyphosate technical daily at 175 mg/kg/day (LOAEL) during GDs 8–20 (an acute-duration oral exposure scenario); the NOAEL was 100 mg/kg/day (EPA 2017b).

Increased severity of basophilia and hypertrophy of acinar cells in parotid and submandibular salivary glands were observed among male and female rats receiving glyphosate from the diet for 13 weeks at 410 and 421 mg/kg/day, respectively; NOAELs were 205 and 213 mg/kg/day, respectively (NTP 1992). Increased severity of basophilia of acinar cells in parotid salivary glands were observed in male and female mice similarly treated at estimated doses of 2,273 and 2,707 mg/kg/day, respectively; NOAELs were 507 and 753 mg/kg/day, respectively (NTP 1992). Thus, rats appear to be much more sensitive than mice to glyphosate treatment-related effects on salivary glands.

Among reliable animal study results, the LOAEL of 350 mg/kg/day for gastrointestinal effects (increased incidence of soft stool and/or diarrhea) in maternal rabbits gavaged daily during GDs 6–27 represents the most sensitive adverse effect from intermediate-duration oral exposure to glyphosate technical (EPA 1992f); the corresponding NOAEL is 175 mg/kg/day (see Table A-2). Incidence and severity data were not available for review. Application of a NOAEL/LOAEL approach using the NOAEL of 175 mg/kg/day as the point of departure and a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) would result in a provisional intermediate-duration oral MRL of 2 mg/kg/day (rounded up from 1.75 mg/kg/day). A provisional intermediate-duration oral MRL was not derived for glyphosate because a provisional intermediate-duration oral MRL of 2 mg/kg/day is higher than the provisional acute- and chronic-duration oral MRL of 1 mg/kg/day. Glyphosate-induced microscopic changes in salivary glands of the rats treated orally for 13 weeks are not considered adequate basis for MRL derivation due to uncertainty regarding the adversity of the effect. However, application of a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to the NOAEL of 205 mg/kg/day for salivary gland changes in male rats administered glyphosate in the diet for 13 weeks would result in a provisional intermediate-duration oral MRL of 2 mg/kg/day. The provisional chronic-duration oral MRL of 1 mg/kg/day for glyphosate is adopted as the provisional intermediate-duration oral MRL because 1 mg/kg/day is considered protective of intermediate-duration oral exposure to glyphosate as well.

Agency Contact (Chemical Manager): Hana R. Pohl, M.D., Ph.D.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Glyphosate technical
CAS Numbers: 1071-83-6
Date: April 2019
Profile Status: Draft for Public Comment
Route: Oral
Duration: Chronic
MRL: 1 mg/kg/day (provisional)
Critical Effect: Inflammation of gastric squamous mucosa
Reference: EPA 1991a, 1991b
Point of Departure: NOAEL of 113 mg/kg/day
Uncertainty Factor: 100
LSE Graph Key: 15
Species: Rat

MRL Summary: A provisional chronic-duration oral MRL of 1 mg/kg/day was derived for glyphosate based on gastrointestinal effects (inflammation of gastric squamous mucosa) observed in female rats administered glyphosate technical in the diet for up to 24 months at an estimated dose of 457 mg/kg/day; the NOAEL was 113 mg/kg/day (EPA 1991a, 1991b). The provisional MRL is based on a NOAEL of 100 mg/kg/day and a total uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

Selection of the Critical Effect: Several chronic-duration oral animal studies were available glyphosate technical (see Table A-3).

Table A-3. NOAELs and LOAELs Identified in Chronic-Duration Oral Studies of Glyphosate Technical

Endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Body weight	13% lower body weight in female rats at treatment week 81	M: 940 F: 457	F: 1,183	EPA 1991a, 1991b
	No effect in rats (highest dose)	M: 31.45 F: 34.02		EPA 1992d
	No effect in rats (highest dose)	M: 1,214 F: 1,498		EPA 2013a
	11–14% lower body weight and body weight gain in rats	300	1,000	EPA 2015c
	No effect in mice (highest dose)	M: 4,945 F: 6,069		EPA 2015a
	No effect in mice (highest dose)	1,000		EPA 2015c
	No effect in dogs (highest dose)	500		EPA 1986a, 1987

Table A-3. NOAELs and LOAELs Identified in Chronic-Duration Oral Studies of Glyphosate Technical

Endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Gastrointestinal	Inflammation of gastric squamous mucosa	M: 940 F: 113	F: 457	EPA 1991a, 1991b
	No effect in rats (highest dose)	M: 31.45 F: 34.02		EPA 1992d
	Increased severity of basophilia and hypertrophy of acinar cells in parotid and mandibular salivary gland in rats	100	300	EPA 2015c
	No effect in mice (highest dose)	M: 4,945 F: 6,069		EPA 2015a
Hematological	No effect in rats (highest dose)	M: 940 F: 1,183		EPA 1991a, 1991b
	No effect in rats (highest dose)	M: 31.45 F: 34.02		EPA 1992d
	No effect in rats (highest dose)	M: 1,214 F: 1,498		EPA 2015c
	No effect in rats (highest dose)	1,000		EPA 2015c
	No effect in mice (highest dose)	M: 4,945 F: 6,069		EPA 2015a
	No effect in dogs (highest dose)	500		EPA 1986a, 1987
Hepatic	No effect in rats (highest dose)	M: 940 F: 1,183		EPA 1991a, 1991b
	No effect in rats (highest dose)	M: 31.45 F: 34.02		EPA 1992d
	Increased serum AP, ALT, bilirubin in male rats; increased serum AP, ALT in female rats	M: 361 F: 437	M: 1,214 F: 1,498	EPA 2015c
	No effect in rats	1,000		EPA 2015c
	Centrilobular hepatocellular necrosis in male rats	M: 835 F: 6,069	M: 4,945	EPA 2015a
	No effect in mice (highest dose)	1,000		EPA 2015c
Renal	Increased specific gravity, decreased pH of urine in male rats	M: 362 F: 1,183	M: 940	EPA 1991a, 1991b
	No effect in rats (highest dose)	M: 31.45 F: 34.02		EPA 1992d
	M: Decreased pH of urine in rats M, F: Papillary necrosis in kidney in rats	M: 361 F:437	M: 1,214 F: 1,498	EPA 2015c
	Decreased pH of urine in male rats	M: 300 F: 1,000	M: 1,000	EPA 2015c
	Renal tubular epithelial basophilia in female mice	M: 4,945 F: 968	F: 6,069	EPA 2015a
	No effect in mice (highest dose)	1,000		EPA 2015c

Table A-3. NOAELs and LOAELs Identified in Chronic-Duration Oral Studies of Glyphosate Technical

Endpoint	Effect	NOAEL (mg/kg/day)	LOAEL (mg/kg/day)	Reference
Ocular	Lens abnormalities in male rats	M: 362 F: 1,183	M: 940	EPA 1991a, 1991b
	No effect in rats	M: 1,214 F: 1,498		EPA 2015c
	No effect in rats	1,000		EPA 2015c
	No effect in dogs (highest dose)	500		EPA 1986a, 1987
Neurological	No effect in rats (highest dose)	M: 1,214 F: 1,498		EPA 2013c

ALT = alanine aminotransferase; AP = alkaline phosphatase; F = female; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level

As shown in Table A-3, gastrointestinal endpoints are the most sensitive to chronic-duration oral exposure of laboratory animals to glyphosate technical. Inflammation of gastric squamous mucosa was observed in female (but not male) rats administered glyphosate technical in the diet for up to 24 months at an estimated dose of 457 mg/kg/day; the NOAEL was 113 mg/kg/day (EPA 1991a, 1991b). Increased severity of cytoplasmic changes in salivary gland cells (basophilia and hypertrophy of acinar cells in parotid and submandibular salivary glands) was reported for rats receiving glyphosate from the diet for 2 years at doses ≥300 mg/kg/day (EPA 2015c). Although salivary gland cytoplasmic changes were noted in rats at doses <300 mg/kg/day as well, the changes were reported to be only of minimal or mild severity; therefore, they are not considered adverse effects. Furthermore, the toxicological significance of the glyphosate treatment-related effects on salivary glands is uncertain. One chronic-duration oral study of male and female mice found no evidence of glyphosate treatment-related gastrointestinal effects at doses as high as 4,945 and 6,069 mg/kg/day, respectively (EPA 1985a, 1985b, 1986b, 1989, 1991c, 1993, 2015a).

Summary of the Principal Study:

EPA. 1991a. June 03, 1991. Memorandum. 40 Page(s). William Dykstra. Toxicology Branch. Glyphosate; 2-Year combined chronic toxicity/carcinogenicity study in Sprague-Dawley rats - List A Pesticide for Reregistration Pages 29-40 removed-registrant data. MRID 416438-01. Tox review 008390. U.S. Environmental Protection Agency. https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/103601/103601-263.pdf. April 10, 2016.

EPA. 1991b. December 13, 1991. Memorandum. 38 Page(s). William Dykstra. Toxicology Branch I. Glyphosate - EPA Registration No. 524-308 - 2-Year chronic feeding/oncogenicity study in rats with technical glyphosate. MRID 416438-01. Tox review 008897. U.S. Environmental Protection Agency. https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/103601/103601-268.pdf. April 10, 2016.

Groups of albino Sprague Dawley rats (60/sex/group) were administered technical glyphosate (96.5% purity) in the diet at target concentrations of 0, 2,000, 8,000, or 20,000 ppm (mean measured concentrations of 0, 1,900, 7,600, and 19,000 ppm, respectively) for up to 24 months. Rats were monitored for survival, clinical signs, food intake, and body weight. Ten rats/sex/dose were subjected to

comprehensive evaluations at 12-month interim sacrifice. Rats were subjected to ophthalmologic examinations prior to the initiation of treatment and twice prior to scheduled terminal sacrifice. Blood and urine samples were collected at 6, 12, 18, and 24 months for hematology, clinical chemistry, and urinalysis. Evaluations of all rats that died or survived until scheduled sacrifice included organ weight determinations (brain, liver, kidneys, testes, epididymides, prostate) and comprehensive gross and histopathologic examinations.

There were no indications of glyphosate-related clinical signs or effects on survival. Mean body weights of all glyphosate-treated male rats were not significantly different from that of controls. Mean body weights and of high-dose female rats were significantly lower than that of controls at weeks 7, 13, 81, and 104 (approximately 3–4% less than that of controls); by week 81, the magnitude of the mean body weight difference between high-dose females and their controls reached 13% (470.6 g versus 543.2 g for controls). There were no significant differences between controls and glyphosate-treated groups regarding food consumption. Based on mean body weight and food consumption data, estimated glyphosate doses to controls and low-, mid-, and high-dose groups were 0, 89, 362, and 940 mg/kg/day, respectively, for the males and 0, 113, 457, and 1,183 mg/kg/day, respectively, for the females.

Glyphosate treatment-related nonneoplastic effects included increased incidence of ocular effects (lens abnormalities), renal effects (increased specific gravity and decreased pH of urine) in high-dose (940 mg/kg/day) male rats, and significantly increased incidence of inflammation of gastric squamous mucosa in female rats at 457 and 1,183 mg/kg/day (incidences of 0/59, 3/60, 9/60 [p=0.0015], and 6/59 [p=0.014] among controls, low-, mid-, and high-dose groups, respectively; statistical significance determined using Fisher's exact test). The high-dose (1,183 mg/kg/day) group of female rats exhibited as much as 13% lower mean body weight at treatment week 81. Relative liver weight was significantly increased in high-dose male rats evaluated at 12 months and terminal sacrifice (13–14% greater than controls); however, histopathologic examinations of liver sections revealed no evidence of significant treatment-related nonneoplastic effects.

Selection of the Point of Departure: A provisional chronic-duration oral MRL can be derived for glyphosate based on incidences of female rats exhibiting gastric lesions in the 2-year dietary study of rats (EPA 1991a, 1991b). Incidences of female rats with gastric lesions were 0/59, 3/60, 9/60, and 6/59 for controls, low-, mid-, and high-dose groups, respectively. All dichotomous models in the Benchmark Dose Modeling Software (BMDS; Version 2.6) were fit to the incidence data for female rats exhibiting inflammation of gastric squamous mucosa. A benchmark response (BMR) of 10% extra risk was applied. None of the models produced adequate fit to the dataset, likely due to 33% lower incidence for the gastric lesion in the high-dose group compared to the mid-dose group. Therefore, a NOAEL/LOAEL approach was employed to derive a provisional chronic-duration oral MRL for glyphosate. The point of departure is the NOAEL of 113 mg/kg/day for gastrointestinal lesions in the female rats of the 2-year dietary study (EPA 1991a, 1991b).

Uncertainty Factor: The NOAEL of 113 mg/kg/day was divided by a total uncertainty factor of 100:

- 10 for animal to human extrapolation
- 10 for human variability

The glyphosate-induced cytoplasmic changes in salivary glands of the chronically-treated rats were not considered for MRL derivation because the toxicological significance of the changes is uncertain. However, consideration of the NOAEL of 100 mg/kg/day (EPA 2015c) as a point of departure, application of a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) would also result in a provisional chronic-duration oral MRL of 1 mg/kg/day.

Other Additional Studies or Pertinent Information: Glyphosate-induced gastrointestinal effects were observed in acute-duration oral studies of rats and rabbits (Adam et al. 1997; EPA 1992e, 2013c, 2017b), intermediate-duration oral studies of rats, mice, and rabbits (EPA 1992a, 1992f; NTP 1992), and chronic-duration oral studies of rats (EPA 1991a, 1991b, 2015c).

Agency Contact (Chemical Manager): Hana R. Pohl, M.D., Ph.D.

APPENDIX B. LITERATURE SEARCH FRAMEWORK FOR GLYPHOSATE

The objective of the toxicological profile is to evaluate the potential for human exposure and the potential health hazards associated with inhalation, oral, or dermal/ocular exposure to glyphosate.

B.1 LITERATURE SEARCH AND SCREEN

A literature search and screen was conducted to identify studies examining health effects, toxicokinetics, mechanisms of action, susceptible populations, biomarkers, chemical interactions, physical and chemical properties, production, use, environmental fate, environmental releases, and environmental and biological monitoring data for glyphosate. ATSDR primarily focused on peer-reviewed articles without publication date or language restrictions. Non-peer-reviewed studies that were considered relevant to the assessment of the health effects of glyphosate have undergone peer review by at least three ATSDR-selected experts who have been screened for conflict of interest. The inclusion criteria used to identify relevant studies examining the health effects of glyphosate are presented in Table B-1.

Table B-1. Inclusion Criteria for the Literature Search and Screen

Health Effects
Species
Human
Laboratory mammals
Route of exposure
Inhalation
Oral
Dermal (or ocular)
Parenteral (these studies will be considered supporting data)
Health outcome
Death
Systemic effects
Body weight effects
Respiratory effects
Cardiovascular effects
Gastrointestinal effects
Hematological effects
Musculoskeletal effects
Hepatic effects
Renal effects
Dermal effects
Ocular effects
Endocrine effects
Immunological effects
Neurological effects
Reproductive effects
Developmental effects
Other noncancer effects

Table B-1. Inclusion Criteria for the Literature Search and Screen

Cancer
Toxicokinetics
Absorption
Distribution
Metabolism
Excretion
PBPK models
Biomarkers
Biomarkers of exposure
Biomarkers of effect
Interactions with other chemicals
Potential for human exposure
Releases to the environment
Air
Water
Soil
Environmental fate
Transport and partitioning
Transformation and degradation
Environmental monitoring
Air
Water
Sediment and soil
Other media
Biomonitoring
General populations
Occupation populations

B.1.1 Literature Search

The following main databases were searched in February 2015 and September 2017:

- PubMed
- National Library of Medicine's TOXLINE
- Scientific and Technical Information Network's TOXCENTER

The search strategy used the chemical names, Chemical Abstracts Service (CAS) numbers, synonyms, and Medical Subject Headings (MeSH) terms for glyphosate. The query strings used for the literature search are presented in Table B-2.

The search was augmented by searching the Toxic Substances Control Act Test Submissions (TSCATS), NTP website, and National Institute of Health Research Portfolio Online Reporting Tools Expenditures and Results (NIH RePORTER) databases using the queries presented in Table B-3. Additional databases were searched in the creation of various tables and figures, such as the TRI Explorer, the Substance

Priority List (SPL) resource page, and other items as needed. Regulations applicable to glyphosate were identified by searching international and U.S. agency websites and documents.

Review articles were identified and used for the purpose of providing background information and identifying additional references. ATSDR also identified reports from the grey literature, which included unpublished research reports, technical reports from government agencies, conference proceedings and abstracts, and theses and dissertations.

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
PubMed 9/2017	("glyphosate"[nm] OR "1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw] OR "Roundup"[tw] OR "34494-03-6"[tw] OR "MON 0459"[tw] OR "40465-66-5"[tw] OR "MON 14420"[tw] OR "MON 8750"[tw] OR "Roundup Hi-Load"[tw] OR "Roundup PRODry"[tw] OR "70393-85-0"[tw] OR "MON 8000"[tw] OR "Monsanto 8000"[tw] OR "Polado"[tw] OR "Trisodium hydrogen bis(N-(phosphonatomethyl)aminoacetate"[tw] OR "39600-42-5"[tw] OR "Glyphosate potassium"[tw] OR "Glyphosate monopotassium salt"[tw] OR "Glyphosate potassium"[tw] OR "Glyphosate-potassium"[tw] OR "Monopotassium glyphosate"[tw] OR "Roundup Attack"[tw] OR "Roundup Energy"[tw] OR "Roundup Maxload"[tw] OR "Roundup Original Max"[tw] OR "Roundup Power Max"[tw] OR "Roundup Ultramax II"[tw] OR "Roundup Weathermax"[tw] OR "Touchdown Forte HiTech"[tw] OR "Transorb R"[tw] OR "Weathermax"[tw] OR "Zapp Qi"[tw] OR "70901-12-1"[tw] OR "Glyphosate-potassium"[tw] OR "Potassium glyphosate"[tw] OR "Potassium N-(phosphonomethyl)glycine"[tw] OR "Uragan Forte"[tw] OR "VisionMAX"[tw] OR "N-(phosphonomethyl)glycine potassium salt"[tw] OR "114370-14-8"[tw] OR "Glyphosate ammonium"[tw] OR "N-(phosphonomethyl)glycine ammonium salt"[tw] OR "69254-40-6"[tw] OR "Glyphosate-diammonium"[tw] OR "Diammonium N-(phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)glycine diammonium salt"[tw]) AND (cancer[sb] OR "neoplasms"[mh] OR "carcinogenicity tests"[mh] OR "carcinogens"[mh] OR "cell division/drug effects"[mh] OR "cell cycle/drug effects"[mh] OR "cell line, tumor/drug effects"[mh] OR "gene expression regulation, neoplastic"[mh] OR "neoplasm proteins/drug effects"[mh] OR "angiogenesis inducing agents"[mh] OR "myelodysplastic-myeloproliferative diseases"[mh] OR cancer*[tw] OR carcinog*[tw] OR carcinom*[tw] OR cocarcinog*[tw] OR lymphoma*[tw] OR neoplas*[tw] OR oncogen*[tw] OR precancer*[tw] OR tumor*[tw] OR tumour*[tw]) AND (2014/02/01 : 3000[dp] OR 2015/02/01 : 3000[mhda] OR 2015/02/01 : 3000[crdat] OR 2015/02/01 : 3000[edat]) ("glyphosate, isopropyl amine salt"[nm] OR "N-(phosphonomethyl)glycine trimethylsulfonium salt"[nm] OR "38641-94-0"[tw] OR "Glyphosate-isopropylammonium"[tw] OR "Glyphosate isopropylamine salt"[tw] OR "Azural AT"[tw] OR "CP 70139"[tw] OR "Fosulen"[tw] OR "Glifosato estrella"[tw] OR "Glycel"[tw] OR "Glycine, N-(phosphonomethyl)-, cmpd with 2-propanamine (1:1)"[tw] OR "Glyfos AU"[tw] OR

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	"Glyfos BIO"[tw] OR "Glyphosate isopropylamine salt"[tw] OR "Glyphosate mono(isopropylamine) salt"[tw] OR "Glyphosate-isopropylammonium"[tw] OR "Glyphosate-mono(isopropylammonium)"[tw] OR "Landmaster"[tw] OR "MON 139"[tw] OR "MON 39"[tw] OR "N-(Phosphonomethyl)glycine isopropylamine salt"[tw] OR "N-(Phosphonomethyl)glycine isopropylammonium salt"[tw] OR "N-(Phosphonomethyl)glycine monoisopropylamine salt"[tw] OR "Nitosorg"[tw] OR "Ron-do"[tw] OR "Utal"[tw] OR "Utal (herbicide)"[tw] OR "Vision (herbicide)"[tw] OR "2-Propanamine, compd, with N-(phosphonomethyl)glycine (1:1)"[tw] OR "Glycine, N-(phosphonomethyl)-, compd. with 2-propanamine (1:1)"[tw] OR "N-(Phosphonomethyl)glycine, compound with 2-propylamine (1:1)"[tw] OR "Isopropylamine glyphosate"[tw] OR "81591-81-3"[tw] OR "Glyphosate-trimesium"[tw] OR "Glyphosphate-trimesium"[tw] OR "Avans 330"[tw] OR "Glyphosate mono(trimethylsulfonium) salt"[tw] OR "Glyphosate trimethylsulfonium salt"[tw] OR "Glyphosate-trimesium"[tw] OR "Medallon"[tw] OR "Ouragan"[tw] OR "R 50224"[tw] OR "SC 0224"[tw] OR "Sulfosate"[tw] OR "Sulphosate"[tw] OR "Touchdown herbicide"[tw] OR "Trimethylsulfonium carboxymethylamino-methylphosphonate"[tw] OR "Trimethylsulfonium glyphosate"[tw] OR "Glycine, N-(phosphonomethyl)-, ion(1-), trimethylsulfonium"[tw] OR "Sulfosate"[tw]) AND (cancer[sb] OR "neoplasms"[mh] OR "carcinogenicity tests"[mh] OR "carcinogens"[mh] OR "cell division/drug effects"[mh] OR "cell cycle/drug effects"[mh] OR "cell line, tumor/drug effects"[mh] OR "gene expression regulation, neoplastic"[mh] OR "neoplasm proteins/drug effects"[mh] OR "angiogenesis inducing agents"[mh] OR "myelodysplastic-myeloproliferative diseases"[mh] OR cancer*[tw] OR carcinog*[tw] OR carcinom*[tw] OR cocarcinog*[tw] OR lymphoma*[tw] OR neoplas*[tw] OR oncogen*[tw] OR precancer*[tw] OR tumor*[tw] OR tumour*[tw]) AND (2014/02/01 : 3000[dp] OR 2015/02/01 : 3000[mhda] OR 2015/02/01 : 3000[crdat] OR 2015/02/01 : 3000[edat])
2/2015	("glyphosate"[nm]) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR (("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh])) OR cancer[sb] OR "pharmacology"[Majr])) OR (("1071-

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) NOT medline[sb])
	("Roundup"[tw] AND (monsanto[tw] OR "antifungal agents"[Pharmacological Action] OR "antifungal agents"[MeSH Terms] OR "antifungal"[tw] OR "anti-fungal"[tw] OR "enzyme inhibitors"[Pharmacological Action] OR "enzyme inhibitors"[MeSH Terms] OR ("enzyme"[tw] AND inhibitor*[tw]) OR "enzyme inhibitors"[tw] OR "enzyme inhibitor"[tw] OR "herbicides"[Pharmacological Action] OR "herbicides"[MeSH Terms] OR "herbicides"[tw] OR "herbicide"[tw] OR "uncoupling agents"[Pharmacological Action] OR "uncoupling agents"[MeSH Terms] OR ("uncoupling"[tw] AND agent*[tw]) OR "uncoupling agent"[tw] OR "uncoupling agents"[tw] OR "pesticides"[mh] OR pesticide*[tw])) NOT (("glyphosate"[nm]) OR ("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR (("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh])) OR cancer[sb] OR "pharmacology"[Majr])) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	"Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) NOT medline[sb]))
	("34494-03-6"[tw] OR "MON 0459"[tw] OR "40465-66-5"[tw] OR "MON 14420"[tw] OR "MON 8750"[tw] OR "Roundup Hi-Load"[tw] OR "Roundup PRODry"[tw] OR "70393-85-0"[tw] OR "MON 8000"[tw] OR "Monsanto 8000"[tw] OR "Polado"[tw] OR "Trisodium hydrogen bis(N-(phosphonatomethyl)aminoacetate"[tw]) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR (("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh])) OR cancer[sb] OR "pharmacology"[Majr])
	("39600-42-5"[tw] OR "Glyphosate potassium"[tw] OR "Glyphosate monopotassium salt"[tw] OR "Glyphosate potassium"[tw] OR "Glyphosate-potassium"[tw] OR "Monopotassium glyphosate"[tw] OR "Roundup Attack"[tw] OR "Roundup Energy"[tw] OR "Roundup Maxload"[tw] OR "Roundup Original Max"[tw] OR "Roundup Power Max"[tw] OR "Roundup Ultramax II"[tw] OR "Roundup Weathermax"[tw] OR "Touchdown Forte HiTech"[tw] OR "Transorb R"[tw] OR "Weathermax"[tw] OR "Zapp Qi"[tw] OR "70901-12-1"[tw] OR "Glyphosate-potassium"[tw] OR "Potassium glyphosate"[tw] OR "Potassium N-(phosphonomethyl)glycine"[tw] OR "Uragan Forte"[tw] OR "VisionMAX"[tw] OR "N-(phosphonomethyl)glycine potassium salt"[tw] OR "114370-14-8"[tw] OR "Glyphosate ammonium"[tw] OR "N-(phosphonomethyl)glycine ammonium salt"[tw] OR "69254-40-6"[tw] OR "Glyphosate-diammonium"[tw] OR "Diammonium N-(phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)glycine diammonium salt"[tw]) NOT (("glyphosate"[nm]) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	"Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR (("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh])) OR cancer[sb] OR "pharmacology"[Majr])) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) NOT medline[sb]) OR ("Roundup"[tw] AND (monsanto[tw] OR "antifungal agents"[Pharmacological Action] OR "antifungal agents"[MeSH Terms] OR "antifungal"[tw] OR "anti-fungal"[tw] OR "enzyme inhibitors"[Pharmacological Action] OR "enzyme inhibitors"[MeSH Terms] OR ("enzyme"[tw] AND inhibitor*[tw]) OR "enzyme inhibitors"[tw] OR "enzyme inhibitor"[tw] OR "herbicides"[Pharmacological Action] OR "herbicides"[MeSH Terms] OR "herbicides"[tw] OR "herbicide"[tw] OR "uncoupling agents"[Pharmacological Action] OR "uncoupling agents"[MeSH Terms] OR ("uncoupling"[tw] AND agent*[tw]) OR "uncoupling agent"[tw] OR "uncoupling agents"[tw] OR "pesticides"[mh] OR pesticide*[tw]))) (((("glyphosate, isopropyl amine salt"[nm]) OR ("N-(phosphonomethyl)glycine trimethylsulfonium salt"[nm])) NOT (("glyphosate"[nm]) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR ci[sh] OR bl[sh] OR

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	cf[sh] OR ur[sh] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR (("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh])) OR cancer[sb] OR "pharmacology"[Majr])) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) NOT medline[sb]) OR ("Roundup"[tw] AND (monsanto[tw] OR "antifungal agents"[Pharmacological Action] OR "antifungal agents"[MeSH Terms] OR "antifungal"[tw] OR "anti-fungal"[tw] OR "enzyme inhibitors"[Pharmacological Action] OR "enzyme inhibitors"[MeSH Terms] OR ("enzyme"[tw] AND inhibitor*[tw]) OR "enzyme inhibitors"[tw] OR "enzyme inhibitor"[tw] OR "herbicides"[Pharmacological Action] OR "herbicides"[MeSH Terms] OR "herbicides"[tw] OR "herbicide"[tw] OR "uncoupling agents"[Pharmacological Action] OR "uncoupling agents"[MeSH Terms] OR ("uncoupling"[tw] AND agent*[tw]) OR "uncoupling agent"[tw] OR "uncoupling agents"[tw] OR "pesticides"[mh] OR pesticide*[tw]))))) OR (("38641-94-0"[tw] OR "Glyphosate-isopropylammonium"[tw] OR "Glyphosate isopropylamine salt"[tw] OR "Azural AT"[tw] OR "CP 70139"[tw] OR "Fosulen"[tw] OR "Glifosato estrella"[tw] OR "Glycel"[tw] OR "Glycine, N-(phosphonomethyl)-, cmpd with 2-propanamine (1:1)"[tw] OR "Glyfos AU"[tw] OR "Glyfos BIO"[tw] OR "Glyphosate isopropylamine salt"[tw] OR "Glyphosate mono(isopropylamine) salt"[tw] OR "Glyphosate-isopropylammonium"[tw] OR "Glyphosate-mono(isopropylammonium)"[tw] OR "Landmaster"[tw] OR "MON 139"[tw] OR "MON 39"[tw] OR "N-(Phosphonomethyl)glycine isopropylamine salt"[tw] OR "N-(Phosphonomethyl)glycine isopropylammonium salt"[tw] OR "N-(Phosphonomethyl)glycine monoisopropylamine salt"[tw] OR "Nitosorg"[tw] OR "Ron-do"[tw] OR "Utal"[tw] OR "Utal (herbicide)"[tw] OR "Vision (herbicide)"[tw] OR "2-Propanamine, compd, with N-(phosphonomethyl)glycine (1:1)"[tw] OR "Glycine, N-(phosphonomethyl)-, compd. with 2-propanamine (1:1)"[tw] OR "N-(Phosphonomethyl)glycine, compound with 2-propylamine (1:1)"[tw] OR "Isopropylamine glyphosate"[tw] OR "81591-81-3"[tw] OR "Glyphosate-trimesium"[tw] OR "Glyphosphate-trimesium"[tw] OR "Avans 330"[tw] OR "Glyphosate mono(trimethylsulfonium) salt"[tw] OR "Glyphosate trimethylsulfonium salt"[tw] OR "Glyphosate-trimesium"[tw] OR "Medallon"[tw] OR "Ouragan"[tw] OR "R 50224"[tw] OR "SC 0224"[tw] OR "Sulfosate"[tw] OR "Sulphosate"[tw] OR "Touchdown herbicide"[tw] OR

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	"Trimethylsulfonium carboxymethylamino-methylphosphonate"[tw] OR "Trimethylsulfonium glyphosate"[tw] OR "Glycine, N-(phosphonomethyl)-, ion(1-), trimethylsulfonium"[tw] OR "Sulfosate"[tw]) NOT (("glyphosate"[nm]) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) AND (to[sh] OR po[sh] OR ae[sh] OR pk[sh] OR (me[sh] AND ("humans"[mh] OR "animals"[mh])) OR ci[sh] OR bl[sh] OR cf[sh] OR ur[sh] OR "environmental exposure"[mh] OR "endocrine system"[mh] OR "hormones, hormone substitutes, and hormone antagonists"[mh] OR "endocrine disruptors"[mh] OR (("Computational biology"[mh] OR "Medical Informatics"[mh] OR Genomics[mh] OR Genome[mh] OR Proteomics[mh] OR Proteome[mh] OR Metabolomics[mh] OR Metabolome[mh] OR Genes[mh] OR "Gene expression"[mh] OR Phenotype[mh] OR genetics[mh] OR genotype[mh] OR Transcriptome[mh] OR ("Systems Biology"[mh] AND ("Environmental Exposure"[mh] OR "Epidemiological Monitoring"[mh] OR analysis[sh])) OR "Transcription, Genetic "[mh] OR "Reverse transcription"[mh] OR "Transcriptional activation"[mh] OR "Transcription factors"[mh] OR ("biosynthesis"[sh] AND (RNA[mh] OR DNA[mh])) OR "RNA, Messenger"[mh] OR "RNA, Transfer"[mh] OR "peptide biosynthesis"[mh] OR "protein biosynthesis"[mh] OR "Reverse Transcriptase Polymerase Chain Reaction"[mh] OR "Base Sequence"[mh] OR "Trans-activators"[mh] OR "Gene Expression Profiling"[mh])) OR cancer[sb] OR "pharmacology"[Majr])) OR (("1071-83-6"[tw] OR "(Carboxymethylamino)methylphosphonic acid"[tw] OR "Carboxymethylaminomethanephosphinic acid"[tw] OR "C-K Yuyos FAV"[tw] OR "CP 67573"[tw] OR "Folusen"[tw] OR "Forsat"[tw] OR "Glialka"[tw] OR "Glifoglex"[tw] OR "Glifosan 747"[tw] OR "gliphosate"[tw] OR "Gliz"[tw] OR "Glyfos"[tw] OR "GlyGran"[tw] OR "Glyphodin A"[tw] OR "Glyphomax"[tw] OR "Glyphosate"[tw] OR "Glyphosphate"[tw] OR "Ground Bio"[tw] OR "Herbatop"[tw] OR "HM 2028"[tw] OR "Kickdown"[tw] OR "Lancer herbicide"[tw] OR "MON 2139"[tw] OR "MON 3539"[tw] OR "MON 6000"[tw] OR "N-(Phosphonomethyl)glycine"[tw] OR "N-(phosphonomethyl)-Glycine"[tw] OR "N-Phosphomethylglycine"[tw] OR "N-Phosphonomethylglycine"[tw] OR "Phorsat"[tw] OR "Phosphonomethylglycine"[tw] OR "Phosphonomethyliminoacetic acid"[tw] OR "Pondmaster"[tw] OR "Rebel Garden"[tw] OR "Roundup Max"[tw] OR "Safal"[tw] OR "Scout herbicide"[tw] OR "Silglif"[tw] OR "yerbimat"[tw]) NOT medline[sb]) OR ("Roundup"[tw] AND (monsanto[tw] OR "antifungal agents"[Pharmacological Action] OR "antifungal agents"[MeSH Terms] OR "antifungal"[tw] OR "anti-fungal"[tw] OR "enzyme inhibitors"[Pharmacological Action] OR "enzyme inhibitors"[MeSH Terms] OR ("enzyme"[tw] AND inhibitor*[tw]) OR "enzyme inhibitors"[tw] OR "enzyme inhibitor"[tw] OR "herbicides"[Pharmacological Action] OR "herbicides"[MeSH Terms] OR "herbicides"[tw] OR "herbicide"[tw] OR "uncoupling agents"[Pharmacological Action] OR "uncoupling agents"[MeSH Terms] OR ("uncoupling"[tw] AND agent*[tw]) OR "uncoupling agent"[tw] OR "uncoupling agents"[tw] OR "pesticides"[mh] OR pesticide*[tw])))))

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
Toxline 9/2017	("lancer herbicide" OR "mon 2139" OR "mon 3539" OR "mon 6000" OR "phorsat" OR "phosphonomethyliminoacetic acid" OR "rebel garden" OR "roundup max" OR "safal" OR "scout herbicide") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	(" (carboxymethylamino) methylphosphonic acid" OR "carboxymethylaminomethanephosphinic acid" OR "c k yuyos fav" OR "cp 67573" OR "folusen" OR "forsat" OR "glialka" OR "glifosan 747" OR "glygran" OR "glyphodin a" OR "glyphomax" OR "ground bio" OR "herbatop" OR "hm 2028" OR "kickdown") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("glifoglex" OR "gliphosate" OR "gliz" OR "glyfos" OR "glyphosate" OR "glyphosphate" OR "n (phosphonomethyl) glycine" OR "n (phosphonomethyl) glycine" OR "n phosphomethylglycine" OR "n phosphonomethylglycine" OR "phosphonomethylglycine" OR "pondmaster" OR "silglif" OR "yerbimat") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	1071-83-6 [rn] AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) [not] PubMed [org] [not] pubdart [org]
	(#7 NOT #4) AND NOT PubMed [org] AND NOT pubdart [org]
	"roundup" AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) [not] PubMed [org] [not] pubdart [org]
	("mon 0459" OR "40465 66 5" OR "mon 14420" OR "mon 8750" OR "roundup hi load" OR "roundup prodry" OR "mon 8000" OR "monsanto 8000" OR "polado" OR "trisodium hydrogen bis (n (phosphonatomethyl) aminoacetate) ") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	(34494-03-6 [rn] OR 70393-85-0 [rn]) AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("glyphosate diammonium" OR "diammonium n (phosphonomethyl) glycine" OR "n (phosphonomethyl) glycine diammonium salt") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("roundup weathermax" OR "touchdown forte hitech" OR "transorb r" OR "weathermax" OR "zapp qi" OR "glyphosate potassium" OR "potassium glyphosate" OR "potassium n (phosphonomethyl) glycine" OR "uragan forte" OR "visionmax" OR "n (phosphonomethyl) glycine potassium salt" OR "glyphosate ammonium" OR "n (phosphonomethyl) glycine ammonium salt") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("glyphosate potassium" OR "glyphosate monopotassium salt" OR "glyphosate potassium" OR "glyphosate potassium" OR "monopotassium glyphosate" OR "roundup attack" OR "roundup energy" OR "roundup maxload" OR "roundup original max" OR "roundup power max" OR "roundup ultramax ii") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	(39600-42-5 [rn] OR 39600-55-0 [rn] OR 39600-56-1 [rn] OR 39600-58-3 [rn] OR 40465-59-6 [rn] OR 40465-64-3 [rn] OR 40465-67-6 [rn] OR 40465-70-1 [rn] OR 40465-90-5 [rn] OR 40465-91-6 [rn] OR 70901-12-1 [rn] OR 114370-14-8 [rn] OR 69254-40-6 [rn]) AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("sulphosate" OR "touchdown herbicide" OR "trimethylsulfonium carboxymethylamino methylphosphonate" OR "trimethylsulfonium glyphosate" OR "glycine n (phosphonomethyl) ion (1) trimethylsulfonium") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("isopropylamine glyphosate" OR "glyphosate trimesium" OR "glyphosphate trimesium" OR "avans 330" OR "glyphosate mono (trimethylsulfonium) salt" OR "glyphosate trimethylsulfonium salt" OR "glyphosate trimesium" OR "medallon" OR "ouragan" OR "r 50224" OR "sc 0224" OR "sulfosate") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("n (phosphonomethyl) glycine monoisopropylamine salt" OR "nitosorg" OR "utal" OR "utal (herbicide) " OR "vision (herbicide) " OR "2 propanamine compd with n (phosphonomethyl) glycine (1 1) " OR "glycine n (phosphonomethyl) compd with 2 propanamine (1 1) " OR "n (phosphonomethyl) glycine compound with 2 propylamine (1 1) ") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	("glyphosate mono (isopropylamine) salt" OR "glyphosate isopropylammonium" OR "glyphosate mono (isopropylammonium) " OR "landmaster" OR "mon 139" OR "mon 39" OR "n (phosphonomethyl) glycine isopropylamine salt" OR "n (phosphonomethyl) glycine isopropylammonium salt") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	("glyphosate isopropylammonium" OR "glyphosate isopropylamine salt" OR "azural at" OR "cp 70139" OR "fosulen" OR "glifosato estrella" OR "glycel" OR "glycine n (phosphonomethyl) cmpd with 2 propanamine (1 1) " OR "glyfos au" OR "glyfos bio" OR "glyphosate isopropylamine salt") AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
	(38641-94-0 [rn] OR 81591-81-3 [rn]) AND 2014:2017 [yr] AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR FEDRIP [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]
2/2015	"Glifoglex" OR "gliphosate" OR "Gliz" OR "Glyfos" OR "Glyphosate" OR "Glyphosphate" OR "N-(Phosphonomethyl)glycine" OR "N-(phosphonomethyl)-Glycine" OR "N-Phosphomethylglycine" OR "N-Phosphonomethylglycine" OR "Phosphonomethylglycine" OR "Pondmaster" OR "Silglif" OR "yerbimat"
	"(Carboxymethylamino)methylphosphonic acid" OR "Carboxymethylaminomethanephosphinic acid" OR "C-K Yuyos FAV" OR "CP 67573" OR "Folusen" OR "Forsat" OR "Glialka" OR "Glifosan 747" OR "GlyGran" OR "Glyphodin A" OR "Glyphomax" OR "Ground Bio" OR "Herbatop" OR "HM 2028" OR "Kickdown"
	"Lancer herbicide" OR "MON 2139" OR "MON 3539" OR "MON 6000" OR "Phorsat" OR "Phosphonomethyliminoacetic acid" OR "Rebel Garden" OR "Roundup Max" OR "Safal" OR "Scout herbicide"
	"roundup"
	34494-03-6[rn] OR 70393-85-0[rn]
	"MON 0459" OR "40465-66-5" OR "MON 14420" OR "MON 8750" OR "Roundup Hi-Load" OR "Roundup PRODry" OR "MON 8000" OR "Monsanto 8000" OR "Polado" OR "Trisodium hydrogen bis(N-(phosphonatomethyl)aminoacetate)"
	39600-42-5[rn] OR 39600-55-0[rn] OR 39600-56-1[rn] OR 39600-58-3[rn] OR 40465-59-6[rn] OR 40465-64-3[rn] OR 40465-67-6[rn] OR 40465-70-1[rn] OR 40465-90-5[rn] OR 40465-91-6[rn] OR 70901-12-1[rn] OR 114370-14-8[rn] OR 69254-40-6[rn]
	"Glyphosate potassium" OR "Glyphosate monopotassium salt" OR "Glyphosate potassium" OR "Glyphosate-potassium" OR "Monopotassium glyphosate" OR "Roundup Attack" OR "Roundup Energy" OR "Roundup Maxload" OR "Roundup Original Max" OR "Roundup Power Max" OR "Roundup Ultramax II"
	"Roundup Weathermax" OR "Touchdown Forte HiTech" OR "Transorb R" OR "Weathermax" OR "Zapp Qi" OR "Glyphosate-potassium" OR "Potassium glyphosate" OR "Potassium N-(phosphonomethyl)glycine" OR "Uragan Forte" OR "VisionMAX" OR "N-

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	(phosphonomethyl)glycine potassium salt" OR "Glyphosate ammonium" OR "N-(phosphonomethyl)glycine ammonium salt"
	"Glyphosate-diammonium" OR "Diammonium N-(phosphonomethyl)glycine" OR "N-(phosphonomethyl)glycine diammonium salt"
	38641-94-0[rn] OR 81591-81-3[rn]
	"Glyphosate-isopropylammonium" OR "Glyphosate isopropylamine salt" OR "Azural AT" OR "CP 70139" OR "Fosulen" OR "Glifosato estrella" OR "Glycel" OR "Glycine, N-(phosphonomethyl)-, cmpd with 2-propanamine (1:1)" OR "Glyfos AU" OR "Glyfos BIO" OR "Glyphosate isopropylamine salt"
	"Glyphosate mono(isopropylamine) salt" OR "Glyphosate-isopropylammonium" OR "Glyphosate-mono(isopropylammonium)" OR "Landmaster" OR "MON 139" OR "MON 39" OR "N-(Phosphonomethyl)glycine isopropylamine salt" OR "N-(Phosphonomethyl)glycine isopropylammonium salt"
	"N-(Phosphonomethyl)glycine monoisopropylamine salt" OR "Nitosorg" OR "Utal" OR "Utal (herbicide)" OR "Vision (herbicide)" OR "2-Propanamine, compd, with N-(phosphonomethyl)glycine (1:1)" OR "Glycine, N-(phosphonomethyl)-, compd. with 2-propanamine (1:1)" OR "N-(Phosphonomethyl)glycine, compound with 2-propylamine (1:1)"
	"Isopropylamine glyphosate" OR "Glyphosate-trimesium" OR "Glyphosphate-trimesium" OR "Avans 330" OR "Glyphosate mono(trimethylsulfonium) salt" OR "Glyphosate trimethylsulfonium salt" OR "Glyphosate-trimesium" OR "Medallon" OR "Ouragan" OR "R 50224" OR "SC 0224" OR "Sulfosate"
	"Sulphosate" OR "Touchdown herbicide" OR "Trimethylsulfonium carboxymethylamino-methylphosphonate" OR "Trimethylsulfonium glyphosate" OR "Glycine, N- N-phosphonemethyl)-, ion(1-), trimethylsulfonium"
Toxcenter 9/2017	L1 9995 SEA 1071-83-6 L2 92 SEA 34494-03-6 OR 40465-66-5 OR 70393-85-0 L3 80 SEA 39600-42-5 OR 39600-55-0 OR 39600-56-1 OR 39600-58-3 OR 40465-59-6 OR 40465-64-3 OR 40465-67-6 OR 40465-70-1 OR 40465-90-5 OR 40465-91-6 L4 101 SEA 70901-12-1 OR 114370-14-8 OR 69254-40-6 L5 2022 SEA 38641-94-0 OR 81591-81-3 L6 10037 SEA L1 OR L2 OR L3 OR L4 L7 6132 SEA L6 NOT (TSCATS/FS OR PATENT/DT) L8 2048 SEA L6 AND (PY>2013 OR ED>=20150201) L9 1260 SEA L7 AND (PY>2013 OR ED>=20150201) L10 751 SEA L5 NOT L6 L11 530 SEA L10 NOT (TSCATS/FS OR PATENT/DT) L12 63 SEA L11 AND (PY>2013 OR ED>=20150201) L13 56 SEA L9 AND (CANCER? OR CARCINOG? OR CARCINOM? OR COCARCINOG? OR LYMPHOMA? OR NEOPLAS? OR ONCOGEN? OR PRECANCER? OR TUMOR? OR TUMOUR?) L14 6 SEA L12 AND (CANCER? OR CARCINOG? OR CARCINOM? OR COCARCINOG? OR LYMPHOMA? OR NEOPLAS? OR ONCOGEN? OR PRECANCER? OR TUMOR?

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	OR TUMOUR?)
	L15 16 SEA L13 AND MEDLINE/FS
	L16 40 SEA L13 NOT L15
	L17 44 DUP REM L15 L16 (12 DUPLICATES REMOVED)
	ANSWERS '1-44' FROM FILE TOXCENTER
	L*** DEL 16 S L13 AND MEDLINE/FS
	L*** DEL 16 S L13 AND MEDLINE/FS
	L18 16 SEA L17
	L*** DEL 40 S L13 NOT L15
	L*** DEL 40 S L13 NOT L15
	L19 28 SEA L17
	L20 28 SEA (L18 OR L19) NOT MEDLINE/FS
	D SCAN L20
	L21 401072 SEA 14 NOT MEDLINE/FS
	L22 6 SEA L14 NOT MEDLINE/FS
	L23 6 DUP REM L22 (0 DUPLICATES REMOVED)
	ANSWERS '1-6' FROM FILE TOXCENTER
	D SCAN L23
	FILE 'MEDLINE' ENTERED AT 19:10:42 ON 14 SEP 2017
	CHARGED TO COST=EH011.10.01
	L24 QUE ACROCHORDON OR ACROCHORDONS OR ADENOMATOSIS OR ADENOMATOUS
	OR ADENOSIS OR AMYLOIDOSES OR AMYLOIDOSIS OR ANAPLASIA OR
	ANGIOENDOTHELIOMATOSIS OR ANGIOMATOSIS OR BUSCHKE-LOWENSTEIN
	OR CANCER OR CANCEROUS OR CANCERS OR CARCINOGEN
	L25 QUE CARCINOGENESIS OR CARCINOGENIC OR CARCINOGENICITY OR
	CARCINOGENS OR CARCINOID OR CARCINOMATOSIS OR CHERUBISM OR CIN
	OR CLL OR COCARCINOGENESIS OR DERMOID OR DYSMYELOPOIESIS OR
	ENCHONDROMATOSIS OR EPIDERMOID OR ERYTHROLEUKAEMIA OR ERYTHROLE
	UKAEMIAS
	L26 QUE ERYTHROLEUKEMIA OR ERYTHROLEUKEMIAS OR ERYTHROPLAKIA OR
	ERYTHROPLAKIAS OR ERYTHROPLASIA OR ESSENTIAL-THROMBOCYTHEMIA
	OR EXOSTOSIS OR FIBROADENOSIS OR FIBROID OR FIBROIDS OR
	FIBROMATOSIS OR GLIOMATOSIS OR GLOMANGIOMATOSIS OR GRANULOMATOS
	IS
	L27 QUE GYNAECOMASTIA OR GYNECOMASTIA OR HEMANGIOMATOSIS OR
	HODGKIN OR HODGKINS OR LEIOMYOMATOSIS OR LEUKAEMIA OR LEUKAEMIA
	S OR LEUKEMIA OR LEUKEMIAS OR LEUKOPLAKIA OR LEUKOPLAKIAS OR
	LEUKOSTASIS OR LIPOBLASTOMATOSIS OR LIPOMATOSIS

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	L28 QUE LYMPHANGIOLEIOMYOMATOSIS OR LYMPHANGIOMATOSIS OR LYMPHANGIO MYOMATOSIS OR LYMPHOPROLIFERATION OR LYMPHOPROLIFERATIONS OR LYMPHOPROLIFERATIVE OR LYMPHOSCINTIGRAPHIC OR LYMPHOSCINTIGRAPH Y OR MACROGLOBULINEMIA OR MACROGLOBULINEMIAS
	L29 QUE MALIGNANCIES OR MALIGNANCY OR MALIGNANT OR MASTOCYTOSIS OR MEIGS-SYNDROME OR MELANOMATOSIS OR MENINGIOMATOSIS OR METAPLASI A OR MICROMETASTASES OR MICROMETASTASIS OR MYCOSIS-FUNGOIDES OR MYELODYSPLASIA OR MYELODYSPLASIAS
	L30 QUE MYELODYSPLASTIC OR MYELOFIBROSIS OR MYELOMATOSIS OR MYELOPROLIFERATION OR MYELOPROLIFERATIONS OR MYELOPROLIFERATIVE OR MYELOSUPPRESSION OR MYOFIBROMATOSIS OR NEOPLASIA OR NEOPLASM OR NEOPLASMS OR NEOPLASTIC OR NEURILEMMOMATOSIS
	L31 QUE NEUROFIBROMATOSIS OR NEURONEVUS OR NONHODGKIN OR NONHODGKIN S OR NONSEMINOMATOUS OR NSCLC OR ONCOGENE-FUSION OR OPSOCLONUS- MYOCLONUS OR PAPILLOMATA OR PAPILLOMATOSIS OR PARANEOPLASTIC OR PEUTZ-JEGHERS OR POLYPOSIS OR PRECANCER
	L32 QUE PRECANCEROUS OR SARCOMATOSIS OR SCHWANNOMATOSIS OR SEMINOMATOUS OR SEZARY-SYNDROME OR STRUMA-OVARII OR TUMOR OR TUMORGENESIS OR TUMORGENIC OR TUMORIGENESIS OR TUMORIGENIC OR TUMOR-MARKER OR TUMOR-MARKERS OR TUMOROGENESIS
	L33 QUE TUMOROGENIC OR TUMORS OR TUMOUR OR TUMOURS OR WALDENSTROM OR WALDENSTROMS OR "5Q-SYNDROME" OR "WAGR SYNDROME" OR (ASCO NOT FUNGI) OR (SENTINEL-LYMPH-NODE NOT BIOPSY)
	L34 QUE L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33
	DIS COST
	FILE 'TOXCENTER' ENTERED AT 19:12:52 ON 14 SEP 2017
	CHARGED TO COST=EH011.10.01
	L47 1 SEA L9 AND ?IOMA
	DIS COST
	L48 26 SEA L9 AND (?AOMA OR ?BOMA OR ?COMA OR ?DOMA OR ?EOMA OR ?FOMA OR ?GOMA OR ?HOMA OR ?IOMA OR ?JOMA OR ?KOMA OR ?LOMA OR ?MOMA

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	OR ?NOMA OR ?OOMA OR ?POMA OR ?QOMA OR ?ROMA OR ?SOMA OR ?TOMA
	OR ?UOMA OR ?VOMA OR ?WOMA)
	L49 0 SEA L9 AND (?XOMA OR ?YOMA OR ?ZOMA OR ?AOMAS OR ?BOMAS OR
	?COMAS OR ?DOMAS OR ?EOMAS OR ?FOMAS OR ?GOMAS OR ?HOMAS OR
	?IOMAS OR ?JOMAS OR ?KOMAS OR ?LOMAS OR ?MOMAS OR ?NOMAS OR
	?OOMAS OR ?POMAS OR ?QOMAS OR ?ROMAS)
	L50 0 SEA L9 AND (?SOMAS OR ?TOMAS OR ?UOMAS OR ?VOMAS OR ?WOMAS OR
	?XOMAS OR ?YOMAS OR ?ZOMAS)
	L51 48 SEA L9 AND L34
	L52 68 SEA L48 OR L49 OR L50 OR L51
	L53 16 SEA L52 NOT L13
	L54 20 SEA L52 AND MEDLINE/FS
	L55 7 SEA L53 AND MEDLINE/FS
	L56 12 DUP REM L53 (4 DUPLICATES REMOVED)
	ANSWERS '1-12' FROM FILE TOXCENTER
	D SCAN L56
	L57 6 SEA L12 AND L34
	L58 2 SEA L12 AND (?AOMA OR ?BOMA OR ?COMA OR ?DOMA OR ?EOMA OR
	?FOMA OR ?GOMA OR ?HOMA OR ?IOMA OR ?JOMA OR ?KOMA OR ?LOMA OR
	?MOMA OR ?NOMA OR ?OOMA OR ?POMA OR ?QOMA OR ?ROMA OR ?SOMA OR
	?TOMA OR ?UOMA OR ?VOMA OR ?WOMA)
	L59 0 SEA L12 AND (?XOMA OR ?YOMA OR ?ZOMA OR ?AOMAS OR ?BOMAS OR
	?COMAS OR ?DOMAS OR ?EOMAS OR ?FOMAS OR ?GOMAS OR ?HOMAS OR
	?IOMAS OR ?JOMAS OR ?KOMAS OR ?LOMAS OR ?MOMAS OR ?NOMAS OR
	?OOMAS OR ?POMAS OR ?QOMAS OR ?ROMAS)
	L60 0 SEA L12 AND (?SOMAS OR ?TOMAS OR ?UOMAS OR ?VOMAS OR ?WOMAS OR
	?XOMAS OR ?YOMAS OR ?ZOMAS)
	L61 8 SEA L57 OR L58
	L62 8 SEA L61 NOT (L13 OR L52)
	L63 7 DUP REM L62 (1 DUPLICATE REMOVED)
	ANSWERS '1-7' FROM FILE TOXCENTER
	D SCAN L63
2/2017	FILE 'TOXCENTER' ENTERED AT 19:21:56 ON 18 FEB 2015
	CHARGED TO COST=EH011.05.01.01
	L1 8342 SEA 1071-83-6
	L2 63 SEA 34494-03-6 OR 40465-66-5 OR 70393-85-0
	L3 8 SEA L2 NOT L1
	L4 53 SEA 39600-42-5 OR 39600-55-0 OR 39600-56-1 OR 39600-58-3 OR 40465-59-6 OR 40465-64-3 OR 40465-67-6 OR 40465-70-1 OR

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	40465-90-5 OR 40465-91-6
	L5 59 SEA 70901-12-1 OR 114370-14-8 OR 69254-40-6
	L6 1828 SEA 38641-94-0 OR 81591-81-3
	L7 8369 SEA L1 OR L2 OR L4 OR L5
	L8 5041 SEA L7 NOT (PATENT/DT OR TSCATS/FS)
	ACT TOXQUERY/Q

	L9 QUE (CHRONIC OR IMMUNOTOX? OR NEUROTOX? OR TOXICOKIN? OR BIOMARKER? OR NEUROLOG?)
	L10 QUE (PHARMACOKIN? OR SUBCHRONIC OR PBPK OR EPIDEMIOLOGY/ST,CT, IT)
	L11 QUE (ACUTE OR SUBACUTE OR LD50# OR LD(W)50 OR LC50# OR LC(W)50)
	L12 QUE (TOXICITY OR ADVERSE OR POISONING)/ST,CT,IT
	L13 QUE (INHAL? OR PULMON? OR NASAL? OR LUNG? OR RESPIR?)
	L14 QUE ((OCCUPATION? OR WORKPLACE? OR WORKER?) AND EXPOS?)
	L15 QUE (ORAL OR ORALLY OR INGEST? OR GAVAGE? OR DIET OR DIETS OR DIETARY OR DRINKING(W)WATER?)
	L16 QUE (MAXIMUM AND CONCENTRATION? AND (ALLOWABLE OR PERMISSIBLE))
	L17 QUE (ABORT? OR ABNORMALIT? OR EMBRYO? OR CLEFT? OR FETUS?)
	L18 QUE (FOETUS? OR FETAL? OR FOETAL? OR FERTIL? OR MALFORM? OR OVUM?)
	L19 QUE (OVA OR OVARY OR PLACENTA? OR PREGNAN? OR PRENATAL?)
	L20 QUE (PERINATAL? OR POSTNATAL? OR REPRODUC? OR STERIL? OR TERATOGEN?)
	L21 QUE (SPERM OR SPERMAC? OR SPERMAG? OR SPERMATI? OR SPERMAS? OR SPERMATOB? OR SPERMATOC? OR SPERMATOG?)
	L22 QUE (SPERMATOI? OR SPERMATOL? OR SPERMATOR? OR SPERMATOX? OR SPERMATOZ? OR SPERMATU? OR SPERMI? OR SPERMO?)
	L23 QUE (NEONAT? OR NEWBORN? OR DEVELOPMENT OR DEVELOPMENTAL?)
	L24 QUE (ENDOCRIN? AND DISRUPT?)
	L25 QUE (ZYGOTE? OR CHILD OR CHILDREN OR ADOLESCEN? OR INFANT?)
	L26 QUE (WEAN? OR OFFSPRING OR AGE(W)FACTOR?)
	L27 QUE (DERMAL? OR DERMIS OR SKIN OR EPIDERM? OR CUTANEOUS?)
	L28 QUE (CARCINOG? OR COCARCINOG? OR CANCER? OR PRECANCER? OR NEOPLAS?)
	L29 QUE (TUMOR? OR TUMOUR? OR ONCOGEN? OR LYMPHOMA? OR CARCINOM?)
	L30 QUE (GENETOX? OR GENOTOX? OR MUTAGEN? OR GENETIC(W)TOXIC?)
	L31 QUE (NEPHROTOX? OR HEPATOTOX?)

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
	L32 QUE (ENDOCRIN? OR ESTROGEN? OR ANDROGEN? OR HORMON?)
	L33 QUE (OCCUPATION? OR WORKER? OR WORKPLACE? OR EPIDEM?)
	L34 QUE L9 OR L10 OR L11 OR L12 OR L13 OR L14 OR L15 OR L16 OR L17 OR L18 OR L19 OR L20 OR L21 OR L22 OR L23 OR L24 OR L25 OR L26 OR L27 OR L28 OR L29 OR L30 OR L31 OR L32 OR L33
	L35 QUE (RAT OR RATS OR MOUSE OR MICE OR GUINEA(W)PIG? OR MURIDAE OR DOG OR DOGS OR RABBIT? OR HAMSTER? OR PIG OR PIGS OR SWINE OR PORCINE OR MONKEY? OR MACAQUE?)
	L36 QUE (MARMOSET? OR FERRET? OR GERBIL? OR RODENT? OR LAGOMORPHA OR BABOON? OR CANINE OR CAT OR CATS OR FELINE OR MURINE)
	L37 QUE L34 OR L35 OR L36
	L38 QUE (HUMAN OR HUMANS OR HOMINIDAE OR MAMMALS OR MAMMAL? OR PRIMATES OR PRIMATE?)
	L39 QUE L37 OR L38
	L40 2675 SEA L8 AND L37
	L41 525 SEA L40 AND MEDLINE/FS
	L42 833 SEA L40 AND BIOSIS/FS
	L43 1263 SEA L40 AND CAPLUS/FS
	L44 0 SEA L40 AND IPA/FS
	L45 54 SEA L40 NOT (L41 OR L42 OR L43)
	L46 2064 DUP REM L41 L42 L43 L45 (611 DUPLICATES REMOVED)
	ANSWERS '1-2064' FROM FILE TOXCENTER
	L*** DEL 525 S L40 AND MEDLINE/FS
	L*** DEL 525 S L40 AND MEDLINE/FS
	L47 525 SEA L46
	L*** DEL 833 S L40 AND BIOSIS/FS
	L*** DEL 833 S L40 AND BIOSIS/FS
	L48 644 SEA L46
	L*** DEL 1263 S L40 AND CAPLUS/FS
	L*** DEL 1263 S L40 AND CAPLUS/FS
	L49 859 SEA L46
	L*** DEL 54 S L40 NOT (L41 OR L42 OR L43)
	L*** DEL 54 S L40 NOT (L41 OR L42 OR L43)
	L50 36 SEA L46
	L51 1539 SEA (L47 OR L48 OR L49 OR L50) NOT MEDLINE/FS
	L52 1532 SEA L51 AND L1
	L53 7 SEA L51 NOT L52
	D SCAN L53
	L54 688 SEA L6 NOT L7
	L55 485 SEA L54 NOT (PATENT/DT OR TSCATS/FS)
	L56 314 SEA L55 AND L37
	L57 0 SEA L56 AND MEDLINE/FS
	L58 85 SEA L56 AND BIOSIS/FS
	L59 218 SEA L56 AND CAPLUS/FS
	L60 1 SEA L56 AND IPA/FS

Table B-2. Database Query Strings Pre-Public Comment Searches

Database search date	Query string
L61	274 DUP REM L56 (40 DUPLICATES REMOVED) ANSWERS '1-274' FROM FILE TOXCENTER D SCAN L52

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
TSCATS[a]	
9/2017; 2/2015	Compounds searched: 1071-83-6; 34494-03-6; 40465-66-5; 70393-85-0; 38641-94-0; 81591-81-3
NTP	
9/2017	glyphosate AND cancer; Limited to 2010-2017
2/2015	"1071-83-6" OR "Glifoglex" OR "gliphosate" OR "Gliz" OR "Glyfos" OR "Glyphosate" OR "Glyphosphate" OR "N-(Phosphonomethyl)glycine" OR "N-(phosphonomethyl)-Glycine" OR "N-Phosphomethylglycine" OR "N-Phosphonomethylglycine" OR "Phosphonomethylglycine" OR "Pondmaster" OR "Silglif" OR "yerbimat" "34494-03-6" OR "40465-66-5" OR "70393-85-0" OR "MON 0459" OR "MON 14420" OR "MON 8750" OR "Roundup Hi-Load" OR "Roundup PRODry" OR "MON 8000" OR "Monsanto 8000" OR "Polado" OR "Trisodium hydrogen bis(N-(phosphonatomethyl)aminoacetate)" "38641-94-0" OR "Glyphosate-isopropylammonium" OR "Glyphosate isopropylamine salt" OR "Azural AT" OR "Buggy" OR "CP 70139" OR "Fosulen" OR "Glifosato estrella" OR "Glycel" OR "Glyfos AU" OR "Glyfos BIO" OR "Glyphosate isopropylamine salt" OR "Glyphosate mono(isopropylamine) salt" OR "Glyphosate-isopropylammonium" OR "Glyphosate-mono(isopropylammonium)" OR "Landmaster" OR "MON 139" OR "MON 39" OR "N-(Phosphonomethyl)glycine isopropylamine salt" OR "N-(Phosphonomethyl)glycine isopropylammonium salt" OR "N-(Phosphonomethyl)glycine monoisopropylamine salt" OR "Nitosorg" OR "Ron-do" OR "Utal" OR "Vision (herbicide)" OR "Roundup" OR "Isopropylamine glyphosate" OR "81591-81-3" OR "Glyphosate-trimesium" OR "Glyphosphate-trimesium" OR "Avans 330" OR "Glyphosate mono(trimethylsulfonium) salt" OR "Glyphosate trimethylsulfonium salt" OR "Glyphosate-trimesium" OR "Medallon" OR "Ouragan" OR "R 50224" OR "SC 0224" OR "Sulfosate" OR "Sulphosate" OR "Touchdown" OR "Trimethylsulfonium carboxymethylamino-methylphosphonate" OR "Trimethylsulfonium glyphosate"
NPIRS 9/2017; 2/2015	PC Codes searched: 417300; 103603; 103613; 103604; 103607; 103601; 128501
NIH RePORTER	
4/2017	Text Search: "Carboxymethylamino)methylphosphonic acid" OR "2-Propanamine, compd. with N-(phosphonomethyl)glycine (1:1)" OR "Avans 330" OR "Azural AT" OR "C-K Yuyos FAV" OR "Carboxymethylaminomethanephosphinic acid" OR "CP 67573" OR "CP 70139" OR "Diammonium N-(phosphonomethyl)glycine" OR "Folusen" OR "Forsat" OR "Fosulen" OR "Glialka" OR "Glifoglex" OR "Glifosan 747" OR "Glifosato estrella" OR "gliphosate" OR "Gliz" OR "Glycel" OR "Glycine, N-(phosphonomethyl)-, cmpd with 2-propanamine (1:1)" OR "Glycine, N-(phosphonomethyl)-, compd. with 2-propanamine (1:1)" OR "Glycine, N-(phosphonomethyl)-, ion(1-), trimethylsulfonium" OR "Glyfos" OR "Glyfos AU" OR "Glyfos BIO" OR "GlyGran" OR "Glyphodin A" OR

Table B-3. Strategies to Augment the Literature Search

Source	Query and number screened when available
	"Glyphomax" OR "Glyphosate" OR "Glyphosphate" OR "Ground Bio" OR "Herbatop" OR "HM 2028" OR "Kickdown" OR "Lancer herbicide" OR "Landmaster" OR "Medallon" OR "MON 0459" OR "MON 139" OR "MON 14420" OR "MON 2139" OR "MON 3539" OR "MON 39" OR "MON 6000" OR "MON 8000" OR "MON 8750" OR "Monsanto 8000" OR "N-(phosphonomethyl)-Glycine" OR "N-(Phosphonomethyl)glycine" OR "N-(phosphonomethyl)glycine ammonium salt" OR "N-(phosphonomethyl)glycine diammonium salt" OR "N-(Phosphonomethyl)glycine isopropylamine salt" OR "N-(Phosphonomethyl)glycine isopropylammonium salt" OR "N-(Phosphonomethyl)glycine monoisopropylamine salt" OR "N-(phosphonomethyl)glycine potassium salt" OR "N-(Phosphonomethyl)glycine, compound with 2-propylamine (1:1)" OR "N-Phosphomethylglycine" OR "N-Phosphonomethylglycine" OR "Nitosorg" OR "Ouragan" OR "Phorsat" OR "Phosphonomethylglycine" OR "Phosphonomethyliminoacetic acid" OR "Polado" OR "Pondmaster" OR "Potassium N-(phosphonomethyl)glycine" OR "R 50224" OR "Rebel Garden" OR "Ron-do" OR "Roundup" OR "Safal" OR "SC 0224" OR "Scout herbicide" OR "Silglif" OR "Sulfosate" OR "Sulphosate" OR "Touchdown Forte HiTech" OR "Touchdown herbicide" OR "Transorb R" OR "Trimethylsulfonium carboxymethylamino-methylphosphonate" OR "Trisodium hydrogen bis(N-(phosphonatomethyl)aminoacetate" OR "Uragan Forte" OR "Utal" OR "Vision herbicide" OR "VisionMAX" OR "Weathermax" OR "yerbimat" OR "Zapp Qi" (Advanced), Search in: Projects AdminIC: All, Fiscal Year: Active Projects, 2017, 2016, 2015, 2014, 2013, 2012
Other	Identified throughout the assessment process

[a]Several versions of the TSCATS database were searched, as needed, by CASRN including TSCATS1 via Toxline (no date limit), TSCATS2 via https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm (date restricted by EPA receipt date), and TSCATS via CDAT (date restricted by 'Mail Received Date Range'), as well as google for recent TSCA submissions.

The 2015 and 2017 results were:

- Number of records identified from PubMed, TOXLINE, and TOXCENTER (after duplicate removal): 5,592
- Number of records identified from other strategies: 211
- Total number of records to undergo literature screening: 5,803

B.1.2 Literature Screening

A two-step process was used to screen the literature search to identify relevant studies on glyphosate:

- Title and abstract screen
- Full text screen

Title and Abstract Screen. Within the reference library, titles and abstracts were screened manually for relevance. Studies that were considered relevant (see Table B-1 for inclusion criteria) were moved to the second step of the literature screening process. Studies were excluded when the title and abstract clearly indicated that the study was not relevant to the toxicological profile.

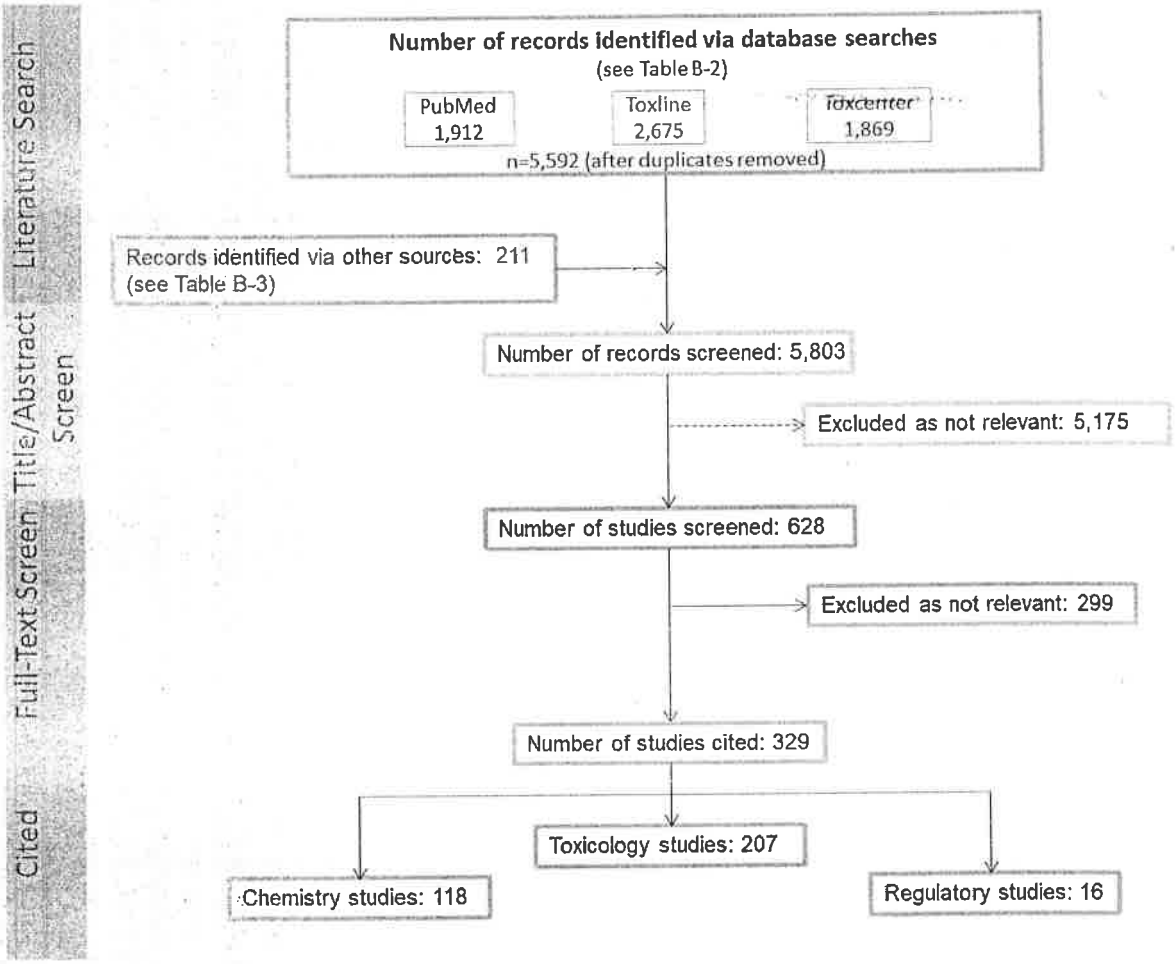
- Number of titles and abstracts screened: 5,803
- Number of studies considered relevant and moved to the next step: 628

Full Text Screen. The second step in the literature screening process was a full text review of individual studies considered relevant in the title and abstract screen step. Each study was reviewed to determine whether it was relevant for inclusion in the toxicological profile.

- Number of studies undergoing full text review: 628
- Total number of studies cited in the profile: 329

A summary of the results of the literature search and screening is presented in Figure B-1.

Figure B-1. February 2015 and September 2017 Literature Search Results and Screen for Glyphosate



APPENDIX C. USER'S GUIDE

Chapter 1. Relevance to Public Health

This chapter provides an overview of U.S. exposures, a summary of health effects based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information, and an overview of the minimal risk levels. This is designed to present interpretive, weight-of-evidence discussions for human health endpoints by addressing the following questions:

1. What effects are known to occur in humans?
2. What effects observed in animals are likely to be of concern to humans?
3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

Minimal Risk Levels (MRLs)

Where sufficient toxicologic information is available, ATSDR derives MRLs for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action, but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans.

MRLs should help physicians and public health officials determine the safety of a community living near a hazardous substance emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Section 1.2, Summary of Health Effects, contains basic information known about the substance. Other sections, such as Section 3.2 Children and Other Populations that are Unusually Susceptible and Section 3.4 Interactions with Other Substances, provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology that the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses (RfDs) for lifetime exposure.

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest no-observed-adverse-effect level (NOAEL) that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a

substance-specific MRL are provided in the footnotes of the levels of significant exposure (LSE) tables that are provided in Chapter 2. Detailed discussions of the MRLs are presented in Appendix A.

Chapter 2. Health Effects

Tables and Figures for Levels of Significant Exposure (LSE)

Tables and figures are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species and MRLs to humans for noncancer endpoints. The LSE tables and figures can be used for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of NOAELs, LOAELs, or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE tables and figures follow. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

TABLE LEGEND

See Sample LSE Table (page C-5)

(1) Route of exposure. One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. Typically, when sufficient data exist, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure (i.e., inhalation, oral, and dermal). LSE figures are limited to the inhalation and oral routes. Not all substances will have data on each route of exposure and will not, therefore, have all five of the tables and figures. Profiles with more than one chemical may have more LSE tables and figures.

(2) Exposure period. Three exposure periods—acute (≤15 days), intermediate (15–364 days), and chronic (≥365 days)—are presented within each relevant route of exposure. In this example, two oral studies of chronic-duration exposure are reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Figure key. Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 51 identified NOAELs and less serious LOAELs (also see the three "51R" data points in sample LSE Figure 2-X).

(4) Species (strain) No./group. The test species (and strain), whether animal or human, are identified in this column. The column also contains information on the number of subjects and sex per group. Chapter 1, Relevance to Public Health, covers the relevance of animal data to human toxicity and Section 3.1, Toxicokinetics, contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(5) Exposure parameters/doses. The duration of the study and exposure regimens are provided in these columns. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 51), rats were orally exposed to "Chemical X" via feed for 2 years. For a

more complete review of the dosing regimen, refer to the appropriate sections of the text or the original reference paper (i.e., Aida et al. 1992).

(6) Parameters monitored. This column lists the parameters used to assess health effects. Parameters monitored could include serum (blood) chemistry (BC), behavioral (BH), biochemical changes (BI), body weight (BW), clinical signs (CS), developmental toxicity (DX), enzyme activity (EA), food intake (FI), fetal toxicity (FX), gross necropsy (GN), hematology (HE), histopathology (HP), lethality (LE), maternal toxicity (MX), organ function (OF), ophthalmology (OP), organ weight (OW), teratogenicity (TG), urinalysis (UR), and water intake (WI).

(7) Endpoint. This column lists the endpoint examined. The major categories of health endpoints included in LSE tables and figures are death, body weight, respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, dermal, ocular, endocrine, immunological, neurological, reproductive, developmental, other noncancer, and cancer. "Other noncancer" refers to any effect (e.g., alterations in blood glucose levels) not covered in these systems. In the example of key number 51, three endpoints (body weight, hematological, and hepatic) were investigated.

(8) NOAEL. A NOAEL is the highest exposure level at which no adverse effects were seen in the organ system studied. The body weight effect reported in key number 51 is a NOAEL at 25.5 mg/kg/day. NOAELs are not reported for cancer and death; with the exception of these two endpoints, this field is left blank if no NOAEL was identified in the study.

(9) LOAEL. A LOAEL is the lowest dose used in the study that caused an adverse health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. Key number 51 reports a less serious LOAEL of 6.1 mg/kg/day for the hepatic system, which was used to derive a chronic exposure, oral MRL of 0.008 mg/kg/day (see footnote "c"). MRLs are not derived from serious LOAELs. A cancer effect level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases. If no LOAEL/CEL values were identified in the study, this field is left blank.

(10) Reference. The complete reference citation is provided in Chapter 8 of the profile.

(11) Footnotes. Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. For example, footnote "c" indicates that the LOAEL of 6.1 mg/kg/day in key number 51 was used to derive an oral MRL of 0.008 mg/kg/day.

FIGURE LEGEND

See Sample LSE Figure (page C-6)

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure period. The same exposure periods appear as in the LSE table. In this example, health effects observed within the chronic exposure period are illustrated.

(14) Endpoint. These are the categories of health effects for which reliable quantitative data exist. The same health effect endpoints appear in the LSE table.

(15) Levels of exposure. Concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) LOAEL. In this example, the half-shaded circle that is designated 51R identifies a LOAEL critical endpoint in the rat upon which a chronic oral exposure MRL is based. The key number 51 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 6.1 mg/kg/day (see entry 51 in the sample LSE table) to the MRL of 0.008 mg/kg/day (see footnote "c" in the sample LSE table).

(17) CEL. Key number 59R is one of studies for which CELs were derived. The diamond symbol refers to a CEL for the test species (rat). The number 59 corresponds to the entry in the LSE table.

(18) Key to LSE figure. The key provides the abbreviations and symbols used in the figure.

Table 2-X. Levels of Significant Exposure to [Chemical X] – Oral [1]

Figure key[a]	Species (strain) No./group	Exposure parameters	Doses (mg/kg/day)	Parameters monitored	Endpoint	NOAEL (mg/kg/day)	Less serious LOAEL (mg/kg/day)	Serious LOAEL (mg/kg/day)	Effect
CHRONIC EXPOSURE									
51	Rat (Wistar) 40 M, 40 F	2 years (F)	M: 0, 6.1, 25.5, 138.0 F: 0, 8.0, 31.7, 168.4	CS, WI, BW, OW, HE, BC, HP	Bd wt	25.5	138.0		Decreased body weight gain in males (23–25%) and females (31–39%)
					Hemato	138.0			
					Hepatic		6.1[c]		Increases in absolute and relative weights at ≥6.1/8.0 mg/kg/day after 12 months of exposure; fatty generation at ≥6.1 mg/kg/day in males and at ≥31.7 mg/kg/day in females, and granulomas in females at 31.7 and 168.4 mg/kg/day after 12, 18, or 24 months of exposure and in males at ≥6.1 mg/kg/day only after 24 months of exposure
Aida et al. 1992									
52	Rat (F344) 78 M	104 weeks (W)	0, 3.9, 20.6, 36.3	CS, BW, FI, BC, OW, HP	Hepatic	36.3			
					Renal	20.6	36.3		Increased incidence of renal tubular cell hyperplasia
					Endocr	36.3			
George et al. 2002									
59	Rat (Wistar) 58M, 58F	Lifetime (W)	M: 0, 90 F: 0, 190	BW, HP	Cancer		190 F		Increased incidence of hepatic neoplastic nodules in females only; no additional description of the tumors was provided
Tumasonis et al. 1985									

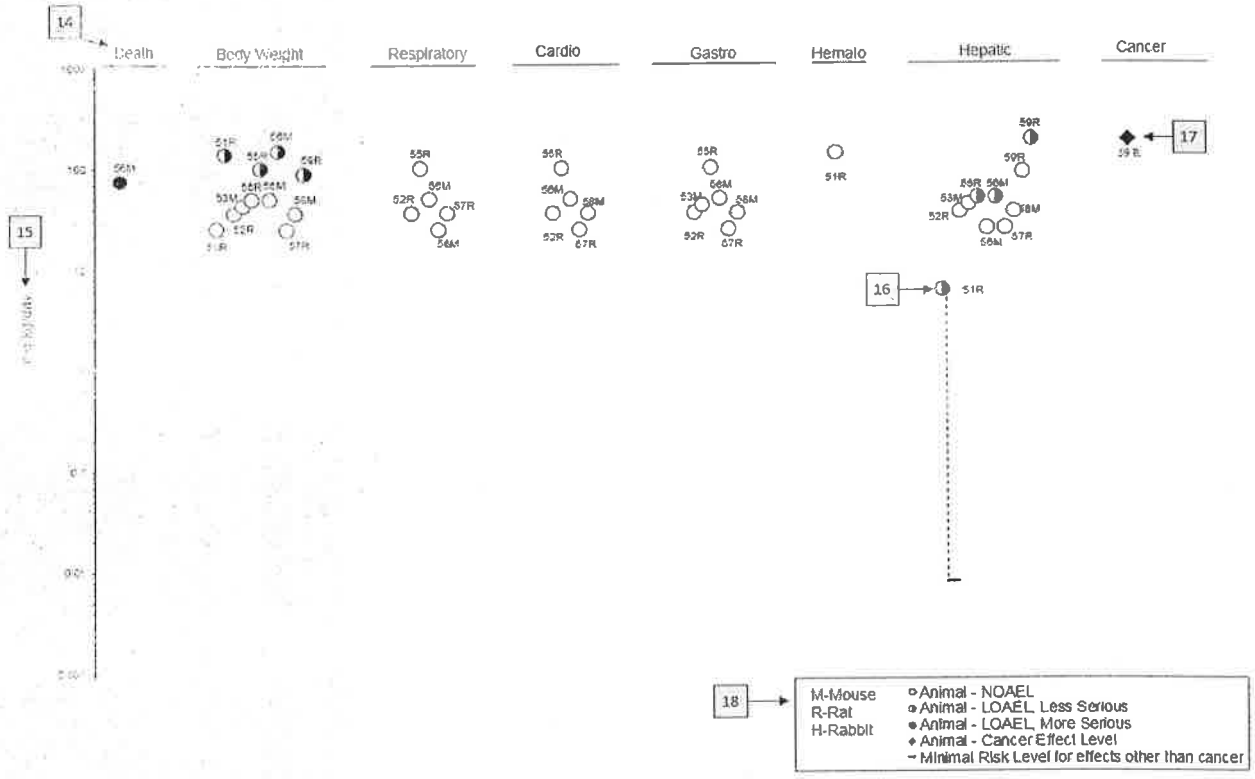
[a]The number corresponds to entries in Figure 2-x.

[11] [b]Used to derive an acute-duration oral minimal risk level (MRL) of 0.1 mg/kg/day based on the $BMDL_{05}$ of 10 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

[c]Used to derive a chronic-duration oral MRL of 0.008 mg/kg/day based on the $BMDL_{10}$ of 0.78 mg/kg/day and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

Figure 2-X. Levels of Significant Exposure to [Chemical X] - Oral

13 → Chronic (≥365 days)



APPENDIX D. QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances may find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Relevance to Public Health: The Relevance to Public Health Section provides an overview of exposure and health effects and evaluates, interprets, and assesses the significance of toxicity data to human health. A table listing minimal risk levels (MRLs) is also included in this chapter.

Chapter 2: Health Effects: Specific health effects identified in both human and animal studies are reported by type of health effect (e.g., death, hepatic, renal, immune, reproductive), route of exposure (e.g., inhalation, oral, dermal), and length of exposure (e.g., acute, intermediate, and chronic).
NOTE: Not all health effects reported in this section are necessarily observed in the clinical setting.

Pediatrics:

Section 3.2 Children and Other Populations that are Unusually Susceptible
Section 3.3 Biomarkers of Exposure and Effect

ATSDR Information Center

Phone: 1-800-CDC-INFO (800-232-4636) or 1-888-232-6348 (TTY)
Internet: http://www.atsdr.cdc.gov

The following additional materials are available online:

Case Studies in Environmental Medicine are self-instructional publications designed to increase primary health care providers' knowledge of a hazardous substance in the environment and to aid in the evaluation of potentially exposed patients (see https://www.atsdr.cdc.gov/csem/csem.html).

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident (see https://www.atsdr.cdc.gov/MHMI/index.asp). Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs™) provide answers to frequently asked questions about toxic substances (see https://www.atsdr.cdc.gov/toxfaqs/Index.asp).

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. Contact: NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015 • Web Page: https://www.cdc.gov/nceh/.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. Contact: NIOSH, 395 E Street, S.W., Suite 9200, Patriots Plaza Building, Washington, DC 20201 • Phone: 202-245-0625 or 1-800-CDC-INFO (800-232-4636) • Web Page: https://www.cdc.gov/niosh/.

The National Institute of Environmental Health Sciences (NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. Contact: NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212 • Web Page: https://www.niehs.nih.gov/.

Clinical Resources (Publicly Available Information)

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. Contact: AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: AOEC@AOEC.ORG • Web Page: http://www.aoec.org/.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. Contact: ACOEM, 25 Northwest Point Boulevard, Suite 700, Elk Grove Village, IL 60007-1030 • Phone: 847-818-1800 • FAX: 847-818-9266 • Web Page: http://www.acoem.org/.

The American College of Medical Toxicology (ACMT) is a nonprofit association of physicians with recognized expertise in medical toxicology. Contact: ACMT, 10645 North Tatum Boulevard, Suite 200-111, Phoenix AZ 85028 • Phone: 844-226-8333 • FAX: 844-226-8333 • Web Page: http://www.acmt.net.

The Pediatric Environmental Health Specialty Units (PEHSUs) is an interconnected system of specialists who respond to questions from public health professionals, clinicians, policy makers, and the public about the impact of environmental factors on the health of children and reproductive-aged adults. Contact information for regional centers can be found at http://pehsu.net/findhelp.html.

The American Association of Poison Control Centers (AAPCC) provide support on the prevention and treatment of poison exposures. Contact: AAPCC, 515 King Street, Suite 510, Alexandria VA 22314 • Phone: 701-894-1858 • Poison Help Line: 1-800-222-1222 • Web Page: http://www.aapcc.org/.

APPENDIX E. GLOSSARY

Absorption—The process by which a substance crosses biological membranes and enters systemic circulation. Absorption can also refer to the taking up of liquids by solids, or of gases by solids or liquids.

Acute Exposure—Exposure to a chemical for a duration of ≤14 days, as specified in the Toxicological Profiles.

Adsorption—The adhesion in an extremely thin layer of molecules (as of gases, solutes, or liquids) to the surfaces of solid bodies or liquids with which they are in contact.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Benchmark Dose (BMD) or Benchmark Concentration (BMC)—is the dose/concentration corresponding to a specific response level estimate using a statistical dose-response model applied to either experimental toxicology or epidemiology data. For example, a BMD_{10} would be the dose corresponding to a 10% benchmark response (BMR). The BMD is determined by modeling the dose-response curve in the region of the dose-response relationship where biologically observable data are feasible. The BMDL or BMCL is the 95% lower confidence limit on the BMD or BMC.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Biomarkers—Indicators signaling events in biologic systems or samples, typically classified as markers of exposure, effect, and susceptibility.

Cancer Effect Level (CEL)—The lowest dose of a chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Case-Control Study—A type of epidemiological study that examines the relationship between a particular outcome (disease or condition) and a variety of potential causative agents (such as toxic chemicals). In a case-control study, a group of people with a specified and well-defined outcome is identified and compared to a similar group of people without the outcome.

Case Report—A report that describes a single individual with a particular disease or exposure. These reports may suggest some potential topics for scientific research, but are not actual research studies.

Case Series—Reports that describe the experience of a small number of individuals with the same disease or exposure. These reports may suggest potential topics for scientific research, but are not actual research studies.

Ceiling Value—A concentration that must not be exceeded.

Chronic Exposure—Exposure to a chemical for ≥365 days, as specified in the Toxicological Profiles.

Clastogen—A substance that causes breaks in chromosomes resulting in addition, deletion, or rearrangement of parts of the chromosome.

Cohort Study—A type of epidemiological study of a specific group or groups of people who have had a common insult (e.g., exposure to an agent suspected of causing disease or a common disease) and are followed forward from exposure to outcome, and who are disease-free at start of follow-up. Often, at least one exposed group is compared to one unexposed group, while in other cohorts, exposure is a continuous variable and analyses are directed towards analyzing an exposure-response coefficient.

Cross-sectional Study—A type of epidemiological study of a group or groups of people that examines the relationship between exposure and outcome to a chemical or to chemicals at a specific point in time.

Data Needs—Substance-specific informational needs that, if met, would reduce the uncertainties of human health risk assessment.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Dose-Response Relationship—The quantitative relationship between the amount of exposure to a toxicant and the incidence of the response or amount of the response.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the effect occurs. Effects include malformations and variations, altered growth, and *in utero* death.

Epidemiology—The investigation of factors that determine the frequency and distribution of disease or other health-related conditions within a defined human population during a specified period.

Excretion—The process by which metabolic waste products are removed from the body.

Genotoxicity—A specific adverse effect on the genome of living cells that, upon the duplication of affected cells, can be expressed as a mutagenic, clastogenic, or carcinogenic event because of specific alteration of the molecular structure of the genome.

Half-life—A measure of rate for the time required to eliminate one-half of a quantity of a chemical from the body or environmental media.

Health Advisory—An estimate of acceptable drinking water levels for a chemical substance derived by EPA and based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—A condition that poses a threat of life or health, or conditions that pose an immediate threat of severe exposure to contaminants that are likely to have adverse cumulative or delayed effects on health.

Immunotoxicity—Adverse effect on the functioning of the immune system that may result from exposure to chemical substances.

Incidence—The ratio of new cases of individuals in a population who develop a specified condition to the total number of individuals in that population who could have developed that condition in a specified time period.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

In Vitro—Isolated from the living organism and artificially maintained, as in a test tube.

In Vivo—Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air that has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{Lo})—The lowest dose of a chemical introduced by a route other than inhalation that has been reported to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical that has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest exposure level of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Lymphoreticular Effects—Represent morphological effects involving lymphatic tissues such as the lymph nodes, spleen, and thymus.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Metabolism—Process in which chemical substances are biotransformed in the body that could result in less toxic and/or readily excreted compounds or produce a biologically active intermediate.

Minimal Risk Level (MRL)—An estimate of daily human exposure to a hazardous substance that is likely to be without an appreciable risk of adverse noncancer health effects over a specified route and duration of exposure.

Modifying Factor (MF)—A value (greater than zero) that is applied to the derivation of a Minimal Risk Level (MRL) to reflect additional concerns about the database that are not covered by the uncertainty factors. The default value for a MF is 1.

Morbidity—The state of being diseased; the morbidity rate is the incidence or prevalence of a disease in a specific population.

Mortality—Death; the mortality rate is a measure of the number of deaths in a population during a specified interval of time.

Mutagen—A substance that causes mutations, which are changes in the DNA sequence of a cell's DNA. Mutations can lead to birth defects, miscarriages, or cancer.

Necropsy—The gross examination of the organs and tissues of a dead body to determine the cause of death or pathological conditions.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to a hazardous substance.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of a chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Although effects may be produced at this dose, they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in *n*-octanol and water, in dilute solution.

Odds Ratio (OR)—A means of measuring the association between an exposure (such as toxic substances and a disease or condition) that represents the best estimate of relative risk (risk as a ratio of the incidence among subjects exposed to a particular risk factor divided by the incidence among subjects who were not exposed to the risk factor). An odds ratio that is greater than 1 is considered to indicate greater risk of disease in the exposed group compared to the unexposed group.

Permissible Exposure Limit (PEL)—An Occupational Safety and Health Administration (OSHA) regulatory limit on the amount or concentration of a substance not to be exceeded in workplace air averaged over any 8-hour work shift of a 40-hour workweek.

Pesticide—General classification of chemicals specifically developed and produced for use in the control of agricultural and public health pests (insects or other organisms harmful to cultivated plants or animals).

Pharmacokinetics—The dynamic behavior of a material in the body, used to predict the fate (disposition) of an exogenous substance in an organism. Utilizing computational techniques, it provides the means of studying the absorption, distribution, metabolism, and excretion of chemicals by the body.

Pharmacokinetic Model—A set of equations that can be used to describe the time course of a parent chemical or metabolite in an animal system. There are two types of pharmacokinetic models: data-based and physiologically-based. A data-based model divides the animal system into a series of compartments, which, in general, do not represent real, identifiable anatomic regions of the body, whereas the physiologically-based model compartments represent real anatomic regions of the body.

Physiologically Based Pharmacodynamic (PBPD) Model—A type of physiologically based dose-response model that quantitatively describes the relationship between target tissue dose and toxic endpoints. These models advance the importance of physiologically based models in that they clearly describe the biological effect (response) produced by the system following exposure to an exogenous substance.

Physiologically Based Pharmacokinetic (PBPK) Model—A type of physiologically based dose-response model that is comprised of a series of compartments representing organs or tissue groups with realistic weights and blood flows. These models require a variety of physiological information, including tissue volumes, blood flow rates to tissues, cardiac output, alveolar ventilation rates, and possibly membrane permeabilities. The models also utilize biochemical information, such as blood:air partition coefficients, and metabolic parameters. PBPK models are also called biologically based tissue dosimetry models.

Prevalence—The number of cases of a disease or condition in a population at one point in time.

Prospective Study—A type of cohort study in which a group is followed over time and the pertinent observations are made on events occurring after the start of the study.

Provisional MRL—A designation applied to an MRL to denote that it is an interim value for public comment. The term "provisional" is removed at release of the finalized document.

Recommended Exposure Limit (REL)—A National Institute for Occupational Safety and Health (NIOSH) time-weighted average (TWA) concentration for up to a 10-hour workday during a 40-hour workweek.

Reference Concentration (RfC)—An estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer health effects during a lifetime. The inhalation RfC is expressed in units of mg/m^3 or ppm.

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily oral exposure of the human population to a potential hazard that is likely to be without risk of deleterious noncancer health effects during a lifetime. The oral RfD is expressed in units of mg/kg/day.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). RQs are (1) $\geq$1 pound or (2) for selected substances, an amount established by regulation either under CERCLA or under Section 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a hazardous substance. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Retrospective Study—A type of cohort study based on a group of persons known to have been exposed at some time in the past. Data are collected from routinely recorded events, up to the time the study is undertaken. Retrospective studies are limited to causal factors that can be ascertained from existing records and/or examining survivors of the cohort.

Risk—The possibility or chance that some adverse effect will result from a given exposure to a hazardous substance.

Risk Factor—An aspect of personal behavior or lifestyle, an environmental exposure, existing health condition, or an inborn or inherited characteristic that is associated with an increased occurrence of disease or other health-related event or condition.

Risk Ratio/Relative Risk—The ratio of the risk among persons with specific risk factors compared to the risk among persons without risk factors. A risk ratio that is greater than 1 indicates greater risk of disease in the exposed group compared to the unexposed group.

Short-Term Exposure Limit (STEL)—A STEL is a 15-minute TWA exposure that should not be exceeded at any time during a workday.

Standardized Mortality Ratio (SMR)—A ratio of the observed number of deaths and the expected number of deaths in a specific standard population.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—An American Conference of Governmental Industrial Hygienists (ACGIH) concentration of a substance to which it is believed that nearly all workers may be repeatedly exposed, day after day, for a working lifetime without adverse effect. The TLV may be expressed as a Time-Weighted Average (TLV-TWA), as a Short-Term Exposure Limit (TLV-STEL), or as a ceiling limit (TLV-C).

Time-Weighted Average (TWA)—An average exposure within a given time period.

Toxicokinetic—The absorption, distribution, metabolism, and elimination of toxic compounds in the living organism.

Toxics Release Inventory (TRI)—The TRI is an EPA program that tracks toxic chemical releases and pollution prevention activities reported by industrial and federal facilities.

Uncertainty Factor (UF)—A factor used in operationally deriving the Minimal Risk Level (MRL), Reference Dose (RfD), or Reference Concentration (RfC) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using lowest-observed-adverse-effect level (LOAEL) data rather than no-observed-adverse-effect level (NOAEL) data. A default for each individual UF is 10; if complete certainty in data exists, a value of 1 can be used; however, a reduced UF of 3 may be used on a case-by-case basis (3 being the approximate logarithmic average of 10 and 1).

Xenobiotic—Any substance that is foreign to the biological system.

APPENDIX F. ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AAPCC	American Association of Poison Control Centers
ACGIH	American Conference of Governmental Industrial Hygienists
ACOEM	American College of Occupational and Environmental Medicine
ACMT	American College of Medical Toxicology
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
AEGL	Acute Exposure Guideline Level
AIC	Akaike's information criterion
AIHA	American Industrial Hygiene Association
ALT	alanine aminotransferase
AOEC	Association of Occupational and Environmental Clinics
AP	alkaline phosphatase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AWQC	Ambient Water Quality Criteria
BCF	bioconcentration factor
BMD/C	benchmark dose or benchmark concentration
BMD_X	dose that produces a X% change in response rate of an adverse effect
$BMDL_X$	95% lower confidence limit on the BMD_X
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
C	centigrade
CAA	Clean Air Act
CAS	Chemical Abstract Services
CDC	Centers for Disease Control and Prevention
CEL	cancer effect level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
Ci	curie
CI	confidence interval
cm	centimeter
CPSC	Consumer Products Safety Commission
CWA	Clean Water Act
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DOD	Department of Defense
DOE	Department of Energy
DWEL	drinking water exposure level
EAFUS	Everything Added to Food in the United States
ECG/EKG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
ERPG	emergency response planning guidelines
F	Fahrenheit
F1	first-filial generation
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act

FR	Federal Register
FSH	follicle stimulating hormone
g	gram
GC	gas chromatography
gd	gestational day
GGT	γ-glutamyl transferase
GRAS	generally recognized as safe
HEC	human equivalent concentration
HED	human equivalent dose
HHS	Department of Health and Human Services
HPLC	high-performance liquid chromatography
HSDB	Hazardous Substance Data Bank
IARC	International Agency for Research on Cancer
IDLH	immediately dangerous to life and health
IRIS	Integrated Risk Information System
Kd	adsorption ratio
kg	kilogram
kkg	kilokilogram; 1 kilokilogram is equivalent to 1,000 kilograms and 1 metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{50}	lethal concentration, 50% kill
LC_{Lo}	lethal concentration, low
LD_{50}	lethal dose, 50% kill
LD_{Lo}	lethal dose, low
LDH	lactic dehydrogenase
LH	luteinizing hormone
LOAEL	lowest-observed-adverse-effect level
LSE	Level of Significant Exposure
LT_{50}	lethal time, 50% kill
m	meter
mCi	millicurie
MCL	maximum contaminant level
MCLG	maximum contaminant level goal
MF	modifying factor
mg	milligram
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
MRL	Minimal Risk Level
MS	mass spectrometry
MSHA	Mine Safety and Health Administration
Mt	metric ton
NAAQS	National Ambient Air Quality Standard
NAS	National Academy of Science
NCEH	National Center for Environmental Health
ND	not detected
ng	nanogram
NHANES	National Health and Nutrition Examination Survey

NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NLM	National Library of Medicine
nm	nanometer
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NR	not reported
NRC	National Research Council
NS	not specified
NTP	National Toxicology Program
OR	odds ratio
OSHA	Occupational Safety and Health Administration
PAC	Protective Action Criteria
PAH	polycyclic aromatic hydrocarbon
PBPD	physiologically based pharmacodynamic
PBPK	physiologically based pharmacokinetic
PEL	permissible exposure limit
PEL-C	permissible exposure limit-ceiling value
pg	picogram
PEHSU	Pediatric Environmental Health Specialty Unit
PND	postnatal day
POD	point of departure
ppb	parts per billion
ppbv	parts per billion by volume
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure level/limit
REL-C	recommended exposure level-ceiling value
RfC	reference concentration
RfD	reference dose
RNA	ribonucleic acid
SARA	Superfund Amendments and Reauthorization Act
SCE	sister chromatid exchange
SD	standard deviation
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase (same as aspartate aminotransferase or AST)
SGPT	serum glutamic pyruvic transaminase (same as alanine aminotransferase or ALT)
SIC	standard industrial classification
SMR	standardized mortality ratio
sRBC	sheep red blood cell
STEL	short term exposure limit
TLV	threshold limit value
TLV-C	threshold limit value-ceiling value
TRI	Toxics Release Inventory
TSCA	Toxic Substances Control Act
TWA	time-weighted average
UF	uncertainty factor
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey

USNRC	U.S. Nuclear Regulatory Commission
VOC	volatile organic compound
WBC	white blood cell
WHO	World Health Organization
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
γ	gamma
δ	delta
µm	micrometer
µg	microgram
q_1^*	cancer slope factor
–	negative
+	positive
(+)	weakly positive result
(–)	weakly negative result

Pesticide residues in food – 2016

Toxicological evaluations

Sponsored jointly by FAO and WHO

Special Session of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

Geneva, Switzerland, 9–13 May 2016

Food and Agriculture Organization of the United Nations

World Health Organization

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TABLE OF CONTENTS

* Evaluated within the periodic review programme of the Codex Committee on Pesticide Residues

319

2016 Special Session of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues

Geneva, 8–13 May 2016

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320

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324

Abbreviations used

AChE	acetylcholinesterase
ACP	acid phosphatase
ADI	acceptable daily intake
AFC	antibody-forming cell
AHS	Agricultural Health Study
AhR	aryl hydrocarbon receptor
ALP	alkaline phosphatase
AMPA	aminomethylphosphonic acid
aOR	adjusted odds ratio
AP	apurinic/apyrimidinic
APG	alkyl polyglucoside
AR	androgen receptor
ARfD	acute reference dose
aRR	adjusted risk ratio
ASDN	androstene-4-ene-3,17-dione
AST	aspartate aminotransferase
AUC	area under the plasma concentration–time curve
AUC_t	area under the concentration versus time–curve calculated up to the last detectable sample
BChE	butyrylcholinesterase
B_{max}	maximum amount of binding
BfR	German Bundesinstitut für Risikobewertung
BMD	benchmark dose
BMD_{10}	estimated benchmark dose for a 10% inhibition
BMD_{15}	estimated benchmark dose for a 15% inhibition
BMD_{20}	estimated benchmark dose for a 20% inhibition
BMD_{30}	estimated benchmark dose for a 30% inhibition
BoNT	botulinum neurotoxin
BUN	blood urea nitrogen
bw	body weight
CA	chromosomal aberrations
CAS	Chemical Abstracts Service
CCPR	Codex Committee on Pesticide Residues
CEBS	Chemical Effects in Biological Systems
cfu	colony-forming unit
ChE	cholinesterase
CHO	Chinese hamster ovary
Ci	curie (1 Ci = 3.7×10^{10} becquerel [Bq])
CI	confidence interval
C_{max}	maximum concentration
CYP	cytochrome P450
CMC	carboxymethylcellulose
CYP	cytochromes P450
2,4-D	2,4-dichlorophenoxyacetic acid
DEL	yeast deletion (assay) DEP diethylphosphoric acid
DETP	diethylphosphorothioic acid
DMSO	dimethyl sulfoxide
DMDTP	dimethyl dithiophosphate
DMP	dimethyl phosphate

322

DMTP	dimethyl thiophosphate
DNA	deoxyribonucleic acid
DPRA	direct peptide reactivity assay
DSB	double strand break
EDSP	Endocrine Disruptor Screening Program
ELISA	enzyme-linked immunosorbent assay
ENDO	endonuclease
EPSPS	5-enolpyruvylshikimate 3-phosphate synthase
eq	equivalent
ER	estrogen receptor
ERTA	estrogen receptor transcriptional activation
F	female
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
F_{2A}	second filial generation, first litter
F_{2B}	second filial generation, second litter
FAO	Food and Agriculture Organization of the United Nations
Fpg	formamidopyrimidine-DNA-glycosylase
FSH	follicle-stimulating hormone
FSTRA	fish short-term reproduction assay
GD	guideline
GGT	gamma-glutamyltransferase
GIT	gastrointestinal tract
GLP	good laboratory practice
GSH	glutathione
Hb	haemoglobin
Hct	haematocrit
Hep2	epidermoid cancer
HepG2	hepatocellular carcinoma
HESS	Hazard Evaluation Support System
HIC	highest ineffective concentration
HPLC	high-performance liquid chromatography
HPLC-EC	high pressure liquid chromatography-electrochemical¬-electrochemical detection
HPLC/MS-MS	high-performance liquid chromatography with mass spectrometry
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HTC	hepatoma cell
IARC	International Agency for Research on Cancer
IC_{50}	median inhibitory concentration
IEDI	international estimated daily intake
IL	interleukin
IP	intraperitoneal
IM	isomalathion
IU	International Unit
IV	intravenous
ISS	Istituto Superiore di Sanità
IW-LED	intensity-weighted lifetime-exposure days
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
K_d	dissociation constant
ke/fd	killed in extremis or found dead
LABC	levator ani plus bulbocavernosus muscle complex
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LDH	lactate dehydrogenase
LEC	lowest effective concentration

LED	lifetime-exposure days
LH	luteinizing hormone
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
M	male
MCH	mean corpuscular haemoglobin
MCV	mean corpuscular volume
MDCA	malathion dicarboxylic acid
MIC	minimum inhibitory concentration
MMC	minimum microbicidal concentration
MMCA	malathion monocarboxylic acid
MN	micronuclei
MN-PCE	micronucleated polychromatic erythrocytes
MOA	mode of action
mRNA	messenger ribonucleic acid
N	sample size
N/A	not applicable
NADH	nicotinamide adenine dinucleotide (reduced)
NADPH	nicotinamide adenine dinucleotide phosphate (reduced)
NB	*nota bene*
NCE	normochromatic erythrocyte
ND	not determined
NHL	non-Hodgkin lymphoma
NI	not investigated
no.	number
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NP	not provided
NR	not reported
N/S	not stated
NS	not specified
NS	not significant
NTE	neuropathy target esterase
NTP	National Toxicology Program
OASIS	Organization for the Advancement of Structured Information Standards
OECD	Organisation for Economic Co-operation and Development
8-OHdG	8-hydroxy-2'-deoxyguanosine
OPPTS	Office of Prevention, Pesticides & Toxic Substances
OR	odds ratio
8-Oxo-dG	8-hydroxy-2'-deoxyguanosine
2-PAM	2-pyridincaldoxime methiodide (in Jenkins, 1988)
2-PAM	pyridine-2-aldoxime methochloride (in Frick et al., 1987, from the 1993 JMPR)
PCE	polychromatic erythrocyte
PDII	primary dermal irritation index
PEG	polyethylene glycol
PHA	phytohaemagglutinin
PND	postnatal day
POE	polyoxyethylene ether
POE-APE	polyoxyethylene ether phosphates – polyoxyethylene alkyl ether phosphate
POEA	polyoxyethyleneamine
POES	polyethoxylated tallow amine
PPAR	peroxisome proliferator-activated receptor
ppb	parts per billion

ppm	parts per million
PWG	Pathology Working Group
PXR	pregnane X receptor
Q	quartile
QSAR	quantitative structure–activity relationships ref. reference
RBA	relative binding affinity
RfD	reference dose
rhCG	recombinant human chorionic gonadotrophin
RNA	ribonucleic acid
ROS	reactive oxygen species RPC_{max} maximum level of response RR risk ratio
rRNA	ribosomal ribonucleic acid
RR	relative risk
rtER	rainbow trout estrogen receptor
S9	9000 × *g* supernatant fraction from liver homogenate
SCE	sister chromatid exchange
SCSA	sperm chromatin structure assay
SD	standard deviation
SDH	succinate dehydrogenase
SDS	sodium dodecyl sulfate
SI	Stimulus Index
SN2	bimolecular nucleophilic substitution
SSB	single strand breaks
StAR	steroidogenic acute regulatory protein
T4	thyroxine
TEPP	tetraethyl pyrophosphate
TK	thymidine kinase
T_{max}	time to reach the maximum concentration
TAF	toxicity adjustment factor
TG	test guideline
Tk	terminal kill
TLC	thin-layer chromatography
TOCP	triorthocresyl phosphate
TP	testosterone propionate
TPA	12-*O*-tetradecanoylphorbol-13-acetate
TSH	thyroid-stimulating hormone
U	enzyme unit
UDS	unscheduled DNA synthesis
USDA	United States Department of Agriculture
USEPA	United States Environmental Protection Agency
UV	ultraviolet
VTG	vitellogenin
v/v	volume per volume
WHO	World Health Organization w/w weight per weight

325

Introduction

The toxicological monographs contained in this volume were prepared by a WHO Core Assessment Group on Pesticide Residues that met with the FAO Panel of Experts on Pesticide Residues in Food and the Environment in a Joint Meeting on Pesticide Residues (JMPR) in Geneva, Switzerland, on 9–13 May 2016.

The three compounds (diazinon, glyphosate and malathion) were evaluated following the recommendation of an electronic task force of the WHO Core Assessment Group on Pesticide Residues that the compounds be re-evaluated due to public health concerns identified by International Agency for Research on Cancer (IARC) and the availability of a significant number of new studies. Reports and other documents resulting from previous Joint Meetings on Pesticide Residues are listed in Annex 1.

The report of the Joint Meeting has been published by the FAO as *FAO Plant Production and Protection Paper 227*. That report contains comments on the compounds considered, acceptable daily intakes and acute reference doses established by the WHO Core Assessment Group. As no residue data were requested, maximum residue levels previously established by the FAO Panel of Experts for these compounds remain unchanged and no monographs on residues were prepared.

The toxicological monographs contained in this volume are based on working papers that were prepared by WHO experts before the 2016 Joint Meeting. A special acknowledgement is made to those experts and to the experts of the Joint Meeting who reviewed early drafts of these working papers.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological properties or toxicity of the compounds included in this volume should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Meeting on Pesticide Residues, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva, Switzerland.

Methodology

Literature search methodology

For each of the 3 compounds under review, the information was collected from 3 sources.

- The individual publications considered by IARC were provided to JMPR.
- The dossiers provided by industry for registration of the compounds in the European Union, the United States of America and Japan were submitted.
- The JMPR experts performed an update of the literature search done by IARC for "cancer", "genotoxicity" and "epidemiological data".

For the articles related to cancer and cancer-mechanisms, the literature search strategy involved performing targeted searches on the agents or major metabolites in the following databases:

1) Google Scholar (http://scholar.google.com/);

2) PubMed (http://www.ncbi.nlm.nih.gov/pubmed);

3) WEB OF SCIENCE (https://apps.webofknowledge.com);

326

4) BioOne (http://www.bioone.org/); and

5) ScienceDirect (http://www.sciencedirect.com/).

A keyword searching strategy was employed, using the keywords and the Boolean Operators (AND, or , and NOT). ([mh] = mesh term; PubMed's controlled vocabulary, [tiab] = text word to be searched in the title or abstract

"Comet Assay"[mh] OR "Germ-line-mutation"[mh] OR "Mutagenesis"[mh] OR "Mutagenicity tests"[mh] OR "Sister-chromatid exchange"[mh] OR "Mutation"[mh] OR

Ames-Assay[tiab] OR Ames-test[tiab] OR Bacterial-Reverse-Mutation-Assay[tiab] OR Clastogen*[tiab] OR DNA-Repair*[tiab] OR Genetic-toxicology[tiab] OR hyperploid[tiab] OR micronucleus-test[tiab] OR tetraploid[tiab] OR Chromosome-aberrations[tiab] OR DNA damage[tiab] OR Mutation[tiab] OR chromosome-translocations[tiab] OR DNA protein crosslinks[tiab] OR DNA-damag*[tiab] OR DNA-inhibit*[tiab] OR Micronuclei[tiab] OR Micronucleus[tiab] OR Mutagens[tiab] OR Strand-break*[tiab] OR Unscheduled-DNA-synthes*[tiab] OR chromosomal-aberration[tiab] OR chromosome-aberration[tiab] OR chromosomal-aberrations[tiab] OR chromosomal-abnormalit*[tiab] OR chromosome-abnormalit*[tiab] OR genotoxic*[tiab] OR Comet-assay[tiab] OR Mutagenic[tiab] OR Mutagenicity[tiab] OR mutations[tiab] OR chromosomal-aberration-test[tiab] OR Sister-chromatid-exchange[tiab]

The search resulted in 157 references for Diazinon–Cancer; 99 for Diazinon–Genotox; 251 for Glyphosate–Cancer; 269 for Glyphosate–Genotox; 227 for Malathion–Cancer; and 182 for Malathion–Genotox.

For epidemiological literature the search was restricted to identifying articles published after the three IARC Monographs were published. The search strategy and results are summarized in the table below.

Search terms	Search engine	Number of hits	Hits after screening for relevance
(diazinon OR glyphosate OR malathion) AND cancer	PubMed (limited to humans; published in the last 5 years)	31	*N* = 2 Koutros et al. (2015); Lerro et al. (2015)
	Scopus (limited to 2014–2016)	28	
(diazinon OR glyphosate OR malathion) AND (NHL OR lymphoma OR leukemia OR "lung cancer" OR "prostate cancer")	PubMed (limited to humans; published in the last 5 years)	11	
	Scopus (limited to 2014–2016)	9	

Methodology of epidemiological studies

The pre-agreed evaluation process and Tier 1 screening criteria used to evaluate epidemiological studies on diazinon, glyphosate and malathion are described in "Section 2.2: Methods for the evaluation of epidemiological evidence for risk assessment" of the JMPR meeting report[1].

Evaluation process of epidemiological evidence for risk assessment for glyphosate, malathion and diazinon

The evaluation process and Tier 1 screening criteria are shown in Fig. 1 below.

[1] Pesticide residues in food 2016: Special session of the joint FAO/WHO meeting on pesticide residues May 2016: Report 2016 (http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/)

327

Fig. 1. Pre-agreed evaluation process and Tier 1 screening criteria

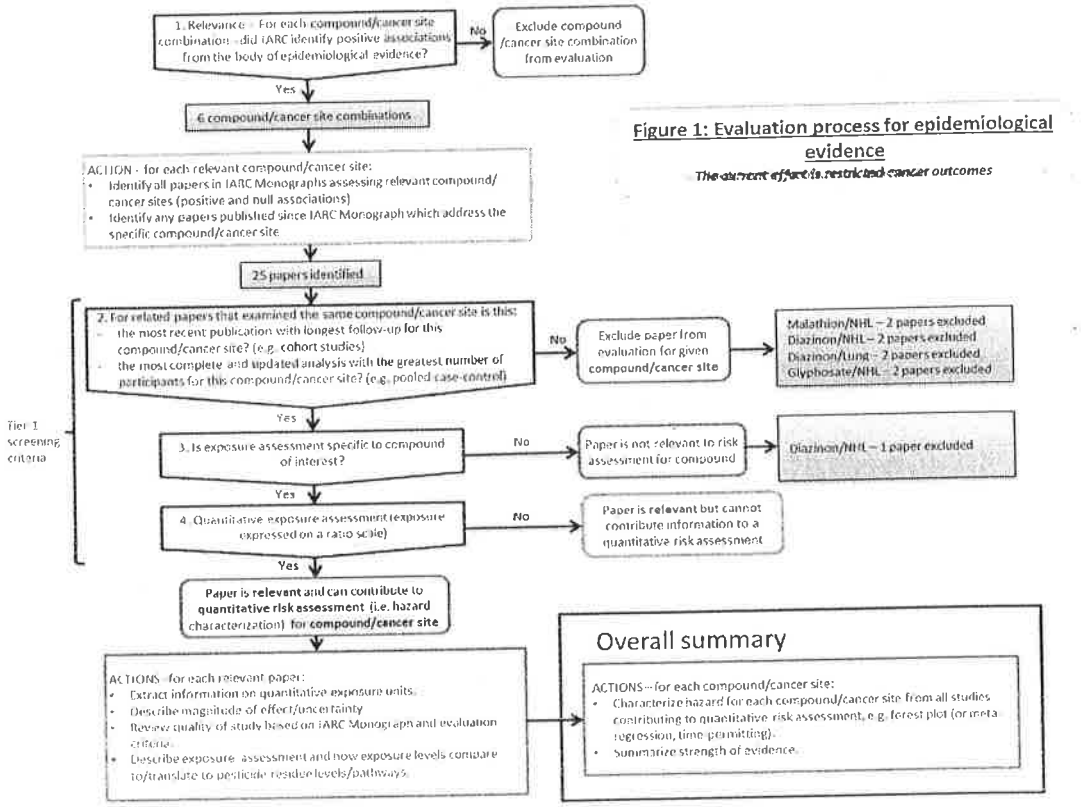

Figure 1: Evaluation process for epidemiological evidence

The current effort is restricted cancer outcomes

(a) Identification of compound/cancer sites and screening of papers

This assessment was restricted to studies of cancer outcomes. The body of epidemiological evidence for non-cancer outcomes was not evaluated; numerous studies have assessed risks for neurodevelopmental, neurodegenerative or reproductive outcomes, among other health outcomes. Restricting the assessment to non-cancer outcomes was partly driven by feasibility reasons: a clinically relevant adverse effect size (or an acceptable level of risk) for a non-cancer outcome must be defined, and the methodologies for hazard identification and characterization based on observational epidemiological findings of non-carcinogenic adverse effects are less well-established than those for cancer (see, for example, Clewell & Crump, 2005; Nachman et al., 2011).

The International Agency for Research on Cancer (IARC) monographs on diazinon, glyphosate and malathion refer to a total of 45 epidemiological studies. Two more recently published studies evaluated at least one of malathion, diazinon or glyphosate in relation to cancer outcomes (Lerro et al., 2015; Koutros et al., 2015). An additional study on prostate cancer (Mills & Yang, 2003), which was not included in the IARC monographs, was also identified.

The 45 publications referred to in the IARC monographs and the three publications since (Mills & Yang, 2003; Lerro et al., 2015; Koutros et al., 2015) covered 48 compound/cancer site combinations. The current evaluation focuses on the 6 compound/cancer site combinations for which IARC identified positive associations from the body of epidemiological evidence, that is, those associations noted in section 6.1 of the monographs, and which underpin IARC's evaluation of limited evidence in humans for the carcinogenicity of malathion, diazinon and glyphosate. The definition for limited evidence of carcinogenicity used by IARC is as follows: "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is

considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence" (IARC, 2015). The 6 compound/cancer site combinations are:

A. Malathion / non-Hodgkin lymphoma (NHL)

B. Malathion / prostate cancer

C. Diazinon / NHL

D. Diazinon / leukaemia

E. Diazinon / lung cancer

F. Glyphosate / NHL

When identifying relevant publications it was noted that there were stand-alone analyses for specific subtypes of NHL (of which there are many). Evaluations of risk for subtypes of NHL were not undertaken separately as there was insufficient evidence (too few studies or small numbers of cases); nor were evaluations of risk undertaken for other haematopoietic and lymphoid tumours, as the positive associations identified by IARC were for total NHL.

There were 26 publications for these 6 compound/cancer site combinations. Seven studies were excluded from at least one evaluation for a given compound/cancer site during Tier 1 screening, either because they were not specific to the pesticide in question; because the publication had been superseded by a later publication on the same cohort and this later publication included longer follow-up time; or because there was a more complete analysis on the same study population with a greater number of participants.

(b) Overview of studies included in evaluation

The IARC monograph on malathion (IARC, 2015) provided an overview of the epidemiological studies which have assessed pesticide exposures and cancer risk. Therefore, only a brief summary (largely based on the IARC monograph) of the studies contributing to the current evaluation is provided here for context.

The Agricultural Health Study is a prospective cohort study of pesticide applicators (predominantly farmers; $n \approx 52\,000$) and their spouses ($n \approx 32\,000$) from Iowa and North Carolina, United States of America, enrolled in 1993–1997. The Study has examined a range of cancer outcomes and published analyses with longer periods of follow-up (e.g. De Roos et al., 2005; Beane Freeman et al., 2005; Koutros et al., 2013; Alavanja et al., 2014; Jones et al., 2015; Lerro et al., 2015). Information on participants' use of 50 pesticides and other determinants of exposure was gathered retrospectively via baseline and two follow-up questionnaires. Cumulative lifetime exposure estimates were calculated. Validation studies have been conducted to assess the reliability and accuracy of exposure intensity scores (a component of the exposure assessment) (Coble et al., 2005; Hines et al., 2008; Thomas et al., 2010). The impact of exposure misclassification in this study was to bias risk estimates towards null (Blair et al., 2011).

The United States Midwest case–control studies are three population-based case–control studies of cancer conducted in Nebraska (Zahm et al., 1990), Iowa and Minnesota (Brown et al., 1990; Cantor et al., 1992) and Kansas (Hoar et al., 1986) that have been pooled (748 cases/2236 controls) to analyse NHL in white males only (Waddell et al., 2001; De Roos et al., 2003; Lee et al., 2004). Information on participants' occupational use of pesticides was gathered retrospectively via a questionnaire. There were some differences in case ascertainment and exposure assessment methods between the three studies. For 39% of the pooled study population, proxy respondents were used (Waddell et al., 2001), for whom recall of specific pesticide use could be problematic and subject to recall bias that may differ for cases and controls. De Roos et al. (2003) used the same study population as Waddell et al. (2001) to perform an extensive evaluation and adjustment for other pesticides.

329

The Cross-Canada Study of Pesticides and Health (CCSPH) is a population-based case–control study of haematopoietic cancers in men diagnosed in 1991–1994 across six Canadian provinces (McDuffie et al., 2001). It includes 517 NHL cases and 1506 controls. A questionnaire was administered by post, followed by a telephone interview for those that reported pesticide exposure of 10 hours/year or more and for a 15% random sample of the remainder. The study was not restricted to pesticide exposure experienced by a specific occupational group (McDuffie et al., 2001). Further analyses stratified by asthma/allergy status – to assess possible effect modification by immune system modulation – have been conducted (Pahwa et al., 2012). The study has a large sample size and detailed information of pesticide exposures; however, the proportion exposed to pesticides was low.

The three sets of studies above were deemed as high quality and highly informative by the IARC Working Group (IARC, 2015).

A number of other case–control studies of pesticide exposure and cancer risk were included in this evaluation: the Florida Pest Control Worker study (Pesatori et al., 1994); nested case–control studies within the United Farm Workers of America cohort study (Mills & Yang, 2003; Mills, Yang & Riordan, 2005); a population-based case–control study of prostate cancer in British Columbia, Canada (Band et al., 2011); and case–control studies of NHL/haematopoietic cancers from Sweden (Hardell et al., 2002; Eriksson et al., 2008) and France (Orsi et al., 2009). The IARC Working Group (IARC, 2015) noted substantial limitations in these studies, either in relation to exposure assessment, scope for and variation in exposure misclassification, lack of detail in the publication, which hindered interpretation, lack of specificity due to high correlations between use of different pesticides, and limited power.

(c) Strengths and limitations of studies included in evaluation

The included studies predominantly examined the occupational pesticide exposures of farmers and other pesticide applicators, with the vast majority of research being on males only. None of the studies assessed exposure via food consumption or ambient exposure from agriculture (e.g. spray drift). The scientific evidence available is therefore limited in its generalizability and the extent to which it can be translated to general population exposure scenarios and levels that would be associated with pesticide residues. Nonetheless, these observational epidemiological studies provide insight into real-world exposure scenarios and allow for observation of the species of interest (humans) over the long follow-up periods relevant to cancer.

The number of high quality studies is relatively small. Typically the number of exposed cases in studies is small, particularly when evaluating specific pesticides, which limits study power.

Relatively few studies have assessed exposure quantitatively, meaning the epidemiological evidence available to inform/establish dose–response relationships is very limited. Exposure misclassification is a potential issue for all studies. This is expected to be largely non-differential for cohort studies (i.e. the Agricultural Health Study), resulting in attenuation of risk estimates. All except one of the studies included are case–control studies, and these may be affected by recall bias, that is, cases and controls recall past pesticide exposure with differing accuracy, leading to differential exposure misclassification that can bias risk estimates either towards or away from the null. As a cohort study, the Agricultural Health Study avoids recall bias.

Given that studies focused on occupational exposures among farmers/pesticide applicators, it is unlikely that they were exposed to only one specific pesticide, so confounding, possible effect modification and additive/multiplicative effects due to coexposures are all concerns. However, many studies were able to adjust risk estimates for other pesticide coexposures, which yields more accurate risk estimates.

There are some issues in terms of comparing studies and evaluating the consistency of evidence overall. Results of studies may appear heterogeneous, but usually there are too few studies to

350

really assess consistency and heterogeneity. Exposure assessment methods and referent groups vary between studies.

Finally, changes in disease classifications (particularly that of NHL) or screening/diagnosis rates (prostate cancer) over time, may limit comparability between studies.

(d) Publication bias

A formal analysis of publication bias was not undertaken because the number of studies (risk estimates from non-overlapping study populations) available were few and it is advised that funnel plot tests for asymmetry be used only where there are at least 10 studies to allow sufficient statistical power to distinguish true asymmetry from chance (Higgins & Green, 2011; Sterne et al., 2011). Other formal objective statistical tests require a larger number of studies, typically at least 30, to achieve sufficient statistical power (Lau et al., 2006). As a result, publication bias cannot be fully excluded. However, given the very considerable resources invested in these types of (large, difficult exposure assessment) studies, it is unlikely that results would go unpublished.

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331

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332

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334

GLYPHOSATE

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Explanation

Glyphosate is the International Organization for Standardization–approved common name for *N*-(phosphonomethyl)glycine (International Union of Pure and Applied Chemistry), with Chemical Abstracts Service (CAS) number 1071-83-6. It is a broad-spectrum systemic herbicide.

Glyphosate was previously evaluated by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for toxicology in 1986, 1997 (evaluation of the metabolite aminomethylphosphonic acid, or AMPA), 2004 and 2011 (evaluation of new plant metabolites in genetically modified maize and soya beans).

Glyphosate was last re-evaluated for toxicology within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR) in 2004. The compound was reviewed by the present Meeting following the recommendation of an electronic task force of the World Health Organization (WHO) Core Assessment Group on Pesticides Residues that it be re-evaluated due to public health concerns identified by the International Agency for Research on Cancer (IARC) and the availability of a significant number of new studies.

The current Meeting evaluated all previously considered toxicological data in addition to new published or unpublished toxicological studies and published epidemiological studies on cancer outcomes. The evaluation of the biochemical aspects and systemic toxicity of glyphosate was based on previous JMPR evaluations, updated as necessary with additional information. The particular focus of the current Meeting was on genotoxicity, carcinogenicity, reproductive and developmental toxicity and epidemiological studies on cancer outcomes. The scope was restricted to the active ingredient.

All critical unpublished studies contained statements of compliance with good laboratory practice (GLP), unless otherwise specified. The studies on human volunteers were conducted in accordance with the principles expressed in the Declaration of Helsinki or equivalent ethical standards.

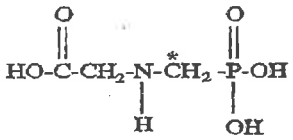
Evaluation for acceptable intake

1. Biochemical aspects

The absorption, distribution, metabolism and excretion of glyphosate was studied in rats following a single oral low dose, a single oral high dose and a single oral low daily dose repeated for 14 days followed by a radioactive dose. In addition, absorption and excretion of glyphosate was studied via intravenous and intraperitoneal administration in rats and intramuscular administration in Rhesus monkeys.

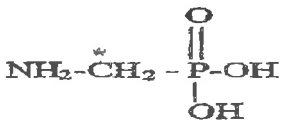
Fig. 1 shows the structure of radiolabelled glyphosate

Fig. 1. Structure of glyphosate – ^{14}C-labelled at the methylene carbon at C1 or C2-glycine carbon



*** Denotes position of ^{14}C label.**

Fig. 2. Structure of aminomethylphosphonic acid (AMPA)



*** Denotes position of ^{14}C label.**

1.1 Absorption, distribution and excretion

(a) Oral route

The excretion and residue levels found by various studies following a single oral dose or repeated oral administration of glyphosate in rats and rabbits are shown in the Table 1.

Table 1. Total elimination and residues of administered radioactivity after single or repeated oral administration of ^{14}C-labelled glyphosate

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
6.7 mg/kg bw Single dose 120 hours	Rat	14–16	35–43	81–85	49–55	0.14–0.65	0.83–1.02	Colvin & Miller[a] (1973a)

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
10 mg/kg bw Single dose 24/48 hours	Rat	17.9/34.0	12.8/12.5	59.3/60.5	80.3/91.2	ND	ND	Davies, (1996a)
10 mg/kg bw Single dose 72 hours	Rat	13	10.6	88.5	88.7	0.59	0.49	Davies, (1996d)
10 mg/kg bw Single dose 7 days	Rat	28.6	22.5	62.4	69.4	0.44	0.31	Ridley & Mirly, (1988)
10 mg/kg bw Repeated dosing 72 hours	Rat	10.6	10.7	86.6	90.7	0.46	0.41	Davies[b] (1996c)
10 mg/kg bw Repeated dosing 7 days	Rat	30.9	23.1	61.0	70.9	0.54	0.35	Ridley & Mirly[b] (1988)
30 mg/kg bw Single dose 168 hours	Rat	29.04	30.71	58.84	56.53	0.62	0.64	Powles, (1992a)
30 mg/kg bw Repeated dosing 168 hours	Rat	34.28	34.63	49.64	46.73	0.96	0.83	Powles, (1992b)
1000 mg/kg bw Single dose 72 hours	Rat	16.7	17.5	89.6	84.5	0.52	0.58	Davies (1996b)
1 000 mg/kg bw Single dose 168 hours	Rat	30.55	22.41	53.27	60.37	0.47	0.40	Powles (1992b)
1 000 mg/kg bw Single dose 7 days	Rat	17.8	14.3	68.9	69.4	0.28	0.24	Ridley & Mirly (1988)
10 mg/kg bw Single dose 168 hours	Rat	22.5	19.4	74.6	84.3	0.33	0.27	McEwen[c] (1995)
10 mg/kg bw Single dose 168 hours	Rat	30.3	29.5	74.7	74.2	0.31	0.39	McEwen[c] (1995)
1 mg/kg bw Single dose 168 hours	Rat	18.4	27.2	72.6	62.4	0.8	1.0	Knowles & Mookherje e (1996[c])
100 mg/kg bw Single dose 168 hours	Rat	39.4	43.1	41.2	42.4	0.8	1.0	Knowles & Mookherje e (1996[c])

Dose administered / No. of doses / Length of study	Species	Total excretion via urine (%)		Total faecal excretion (%)		Total tissue and residual carcass residues (%)		Reference
		Males	Females	Males	Females	Males	Females	
5.7–8.8 mg/kg bw Single dose 120 hours	Rabbit	7–11	ND	80–97	ND	0.1–1.2	ND	Colvin & Miller[a] (1973c)

bw: body weight; ND: not determined; no. number

[a] Glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon or at the C2-glycine carbon.

[b] Groups of male and female rats were given 14 consecutive daily oral doses of 10 mg/kg bw of unlabelled glyphosate followed by a single oral dose 10 mg/kg bw of [^{14}C]glyphosate.

[c] Residual activity in carcass only.

The excretion and residue levels found by various studies following single intraperitoneal, intravenous or intramuscular administration in rats and Rhesus monkeys are shown in Table 2.

Table 2. Residues of administered ^{14}C-labelled glyphosate[a] after single dose administration

Dose / Means of administration / Length of observation	Species	Percentage of administered dose (%)						Reference
		Total excretion via urine		Total faecal excretion		Total tissue and residual carcass residues		
		Males	Females	Males	Females	Males	Females	
6.7 mg/kg bw Intraperitoneal 120 hours	Rat	82–90	ND	6–14	ND	< 1	ND	Colvin & Miller[a] (1973a)
10 mg/kg bw Intravenous 7 days	Rat	79.0	74.5	4.65	8.3	1.27	1.09	Ridley & Mirly (1988)
30 mg/kg bw Intravenous 168 hours	Rat	85.98	84.18	3.42	1.48	1.35	1.09	Powles (1992b)
4 mg Intramuscular 7 days	Monkey	89.9	ND	ND	ND	ND	ND	Maibach (1983)

bw: body weight; ND: not determined

[a] Glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon or at the C2-glycine carbon.

Rats

In a pre-GLP study, aqueous solutions of glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon and at the C2-glycine carbon were administered to Wistar rats by gavage. The radiochemical purity of the labelled materials used were 95% and higher for ^{14}C-methylene glyphosate, ^{14}C-C1-glycine glyphosate and ^{14}C-C2-glycine glyphosate. For the first series of experiments, eight male and four female rats were fasted for four hours and then administered, by gavage, aqueous solutions of [^{14}C]glyphosate at a dose level of 6.7 mg/kg body weight (bw). Two male rats and one female rat were administered ^{14}C-methylene glyphosate, three male rats and one female rat were administered ^{14}C-C1-glycine glyphosate, and three male rats and two female rats were administered ^{14}C-C2-glycine glyphosate. In a second series of experiments, three treatment groups of

three male rats each were dosed separately, via intraperitoneal injection, with ^{14}C-methylene glyphosate (2.33 mg/kg bw), ^{14}C-C1-glycine glyphosate (2.91 mg/kg bw) and ^{14}C-C2-glycine glyphosate (3.63 mg/kg bw). In a third series of experiments designed to determine the gross distribution of plant-derived metabolites of glyphosate, aqueous extracts of soybeans grown in hydroponic solutions of [^{14}C]glyphosate were administered orally to rats. The extracts were obtained from soybean plants, which had been cultured for 4 weeks in separate hydroponic media containing the three forms of [^{14}C]glyphosate. Treatment groups composed of three male rats each for each type of radiolabelled material were dosed separately with the aqueous extracts of the roots of soybeans. A fourth treatment group of three male rats was also dosed with the aqueous extract of the aerial portion of soybean plants grown in hydroponic media containing ^{14}C-methylene glyphosate.

Approximately 94–98% of the [^{14}C]glyphosate orally administered to male rats was excreted in urine and faeces within 48 hours of administration. Approximately 15% of the dose was excreted in the urine within 120 hours of administration, with most of the remainder excreted in the faeces (81%–85%). Of the [^{14}C]glyphosate absorbed through the gut, only very small amounts were catabolized. The percentage of administered radioactivity recovered as expired $^{14}CO_2$ was 0.5%. Tissue retention 120 hours post-administration was less than 1% of the dose for the three ^{14}C-labelled forms of glyphosate.

The percentage of radioactivity excreted by female rats after oral administration of [^{14}C]glyphosate was 82–84% at 48 hours and 91–93% 120 hours. Between 34% and 40% of the administered radioactivity was excreted in the urine within 120 hours, with most of the remainder excreted in the faeces (49–55%). The levels of exhaled $^{14}CO_2$ were also slightly higher for female than male rats, as were carcass retentions. For female rats, the percentage of administered radioactivity recovered as expired $^{14}CO_2$ was 0.72%. Tissue retention at 120 hours was approximately 1% for the three ^{14}C-labelled forms of glyphosate. For both sexes, the order of retention of radioactivity in tissues 120 days post-administration was similar, although the female tissues contained higher concentrations. The highest concentrations of radioactivity were found in the liver, kidney and gut, but in all cases these were 0.20 parts per million (ppm) or less on a fresh-weight basis.

About 74–78% of the dose of [^{14}C]glyphosate administered to male rats via intraperitoneal injection was excreted in the urine within 12 hours. At 96 hours post-administration, total urinary excretion ranged from 81–90% of the administered dose. Faecal excretion ranged from 6–14% of the administered radioactivity within 96 hours and strongly suggests that [^{14}C]glyphosate is also eliminated via the bile. The percentage of radioactivity recovered as expired $^{14}CO_2$ was slightly greater than that following oral administration, but for all three radiolabels was less than 1% of the administered dose. Tissue retention was also greater in female than in male rats after oral administration, but in all cases was 1% or less of the administered dose.

When extracts of soybeans grown in hydroponic solutions of [^{14}C]glyphosate were orally administered to male rats, 96–99% of the administered radioactivity was excreted in the faeces and urine within 120 hours. The exception were the rats dosed with extracts of soybean roots from plants treated with ^{14}C-C2-glycine glyphosate, for which only 76% of the administered dose was found in the excreta. The relatively high tissue retention (5.19% and 1.86% of the administered dose) and $^{14}CO_2$ expiration (3.67% and 3.49% of the administered dose) by rats administered extracts of roots from plants treated with ^{14}C-C2-glycine glyphosate and the extracts of the aerial portion of plants treated with [^{14}C]methylene glyphosate was attributed to the metabolism of natural plant products since the radioactivity in these extracts was due to 30% and 10% natural products, respectively (Colvin & Miller, 1973a).

In a pre-GLP study, the accumulation and depletion of glyphosate was investigated by the daily administration of feed containing 0, 1, 10 and 100 ppm of [^{14}C]glyphosate to Wistar rats (15/sex per dose) for 14 days, followed by a 10-day depuration period on a control ration. Tissue residues were measured after 2, 6, 10 and 14 days on dosed feed and 1, 3, 6 and 10 days after withdrawal from the dosed feed. The excretion of ingested [^{14}C]glyphosate in faeces and urine were determined daily.

340

Body and organ weights indicated that the continuous administration of feed containing 1, 10 and 100 ppm of glyphosate for 14 days had no detrimental effect on the growth or relative organ size of rats. Of the [^{14}C]glyphosate ingested, 8.3–10.5% of the daily intake was excreted in the urine. The combined urinary and faecal excretion of radioactivity was approximately equal to the total intake of [^{14}C]glyphosate after 6 days, indicating that a plateau had been reached. By day 4 of dosing, radioactivity in the urine plus faeces exceeded 90% of the cumulative intake, and by the end of the 14-day dosing period the combined excretion of radioactivity was 96, 115 and 93% of the cumulative intake of the 1, 10 and 100 ppm dosing levels, respectively. Since the amount of radioactivity excreted was directly proportional to the intake, the elimination kinetic of [^{14}C]glyphosate could be described as a first-order process, precluding the potential of unlimited accumulation. Most tissues reached maximum [^{14}C]glyphosate residue levels during the dosing period in 10 days or less. There was a modest cumulative effect in the body as a result of chronic [^{14}C]glyphosate administration, but the effect was not localized in a single tissue type or organ system. The order of decreasing tissue propensity for [^{14}C]glyphosate, on a fresh-weight basis, was kidney, spleen, fat, liver, ovaries, heart, muscle, brain and testes. On a dry-weight base the order was spleen, kidney, ovaries, heart, liver, testes, fat, brain and muscle. Accumulation of [^{14}C]glyphosate in muscle tissue was very low on either a fresh- or dry-weight basis, indicating a very low propensity for accumulation. The residues in the tissues were reversibly bound and began to deplete as soon as the dosed feed was withdrawn (Colvin & Miller, 1973b).

Seven different test groups of Sprague Dawley (Crl:CD[SD]BR) rats, each with an equal number of males and females, were dosed with [^{14}C]glyphosate labelled in the methylene position between the nitrogen and phosphorous atoms (radiochemical purity ≥ 98%). Single oral doses (10 and 1000 mg/kg bw) were administered by gastric intubation, and intravenous doses (10 mg/kg bw) were injected into the lateral tail vein. Another group of five male and five female rats was treated with unlabelled glyphosate as 14 consecutive oral doses at 10 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 10 mg/kg bw. Blood, urine and faeces were sampled at various time points. At the end the study, the animals were terminated and different tissues as well as the carcass analysed for radioactivity.

The distribution of radioactivity in the excreta and the tissue samples are summarized in Table 3.

Table 3. Recovery of radioactivity as a percentage of the administered ^{14}C-labelled glyphosate dose

	Per cent of administered radioactive dose (%)							
	Single IV dose 10 mg/kg bw		Single oral dose 10 mg/kg bw		Repeated oral dose 10 mg/kg bw		Single oral dose 1 000 mg/kg bw	
Excreta/tissue	Males	Females	Males	Females	Males	Females	Males	Females
Urine	79.0	74.5	28.6	22.5	30.9	23.1	17.8	14.3
Faeces	4.65	8.30	62.4	69.4	61.0	70.9	68.9	69.4
Organs/tissues	0.09	0.05	0.05	0.02	0.05	0.03	0.04	0.03
Residual carcass	1.18	1.04	0.40	0.29	0.50	0.32	0.25	0.21
Gastrointestinal contents	0.04	0.04	0.02	0.01	0.01	0.01	0.03	0.04
Cage wash	0.89	1.30	1.30	1.96	0.82	1.96	3.86	8.00
Total recovery[a]	86.0	85.3	92.8	94.2	93.3	96.3	90.9	92.1

bw: body weight; IV: intravenous

[a] Total recovery is the mean of individual animal data.

Source: Ridley & Mirly (1988)

The major route of elimination of an oral dose of ^{14}C-labelled glyphosate at 10 mg/kg body bw was faeces. After a 7-day elimination period, the faeces contained 62.4% and 69.4% of the administered dose for males and females, respectively. The majority of the remaining radioactivity, 28.6% of the dose for the males and 22.5% of the dose for females, was excreted in the urine. More of the administered dose remained in the organs, tissues and residual carcasses of the males than of the females, although the overall amount of retained radioactivity was very low (< 0.5% of the administered dose). The tissue with the highest concentrations of radioactivity was bone, with 0.552 ppm and 0.313 ppm found for the males and females, respectively.

For the test group orally dosed at 1000 mg/kg bw, 68.9% and 69.4% of the administered dose was excreted in the faeces and 17.8% and 14.3% was excreted in the urine of the male and female rats, respectively. Very low levels (< 0.4%) of the administered dose remained in the gastrointestinal contents, residual carcasses, organs and tissues 7 days after dosing. The tissues showing more than 1.0 ppm of radioactivity were the liver, kidney, spleen, lung, stomach, small intestines, bone and residual carcass. Bone retained the greatest amount of radioactivity, 30.6 ppm and 19.7 ppm for the males and females, respectively.

For the test group treated with 14 daily doses of non-labelled glyphosate at 10 mg/kg prior to receiving a single oral dose of labelled glyphosate at 10 mg/kg bw, males excreted 61.0% and 30.9% and females 70.9% and 23.1% of the dose in the faeces and urine, respectively. Very low levels (< 0.7%) of the administered dose remained in the gastrointestinal contents, residual carcasses, organs and tissues 7 days after dosing. Again, bone was the tissue with the highest concentration of radioactivity, containing 0.748 and 0.462 ppm glyphosate equivalents for male and female rats, respectively.

The half-lives of the α and β elimination phases were 5.9–6.2 hours and 79–106 hours, respectively, following a single oral dose of 10 mg/kg bw. In the 1000 mg/kg bw dosed group, the α phase was comparable to 10 mg/kg bw group, but the β phase was found to be 181–337 hours. Comparison of the area under the curves of plots of radioactivity levels in the blood versus time for the two groups indicated that the orally administered glyphosate was 30–35% absorbed. These values are in good agreement with the absorption values of 30–36% found by dividing the per cent urinary excretion of administered radioactivity for the group dosed orally at 10 mg/kg bw by the per cent urinary excretion of administered radioactivity from the group dosed intravenously at 10 mg/kg bw. The results of this study demonstrate that glyphosate is poorly absorbed and rapidly eliminated after a single oral dose at 10 or 1000 mg/kg bw (Ridley & Mirly, 1988).

In a preliminary study of absorption and distribution, male Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material = 98.6%; radiochemical purity = 94.3–97.4%) as a single oral dose at 30 mg/kg bw in 0.9% saline by gavage. Blood samples were taken from the tail vein of three animals periodically between 0.5 and 48 hours after dosing. Additional animals were terminated 4, 10 and 24 hours after dosing, and the tissue distribution of radioactivity was investigated by whole-body autoradiography.

Low levels of radioactivity were detected in plasma. Maximum plasma concentrations (C_{max}) reached within 4 hours were 1.769, 1.137 and 0.705 µg eq/mL. Thereafter, plasma levels decayed exponentially to non-detectable levels 12 hours post dose. The elimination half-lives were 6.196 hours and 12.35 hours for two animals. A value could not be obtained for the third animal. The concentration of radioactivity was highest after 10 hours, with the highest concentrations in bone, bone marrow, cartilage, parts of the gastrointestinal tract, kidney, urinary tract and nasal mucosa. The highest concentrations within bone were associated with the epiphyses. Lower concentrations were found in a number of other tissues. Twenty-four hours after dosing, tissue concentrations of radioactivity were negligible in all tissues except bone, bone marrow, parts of the gastrointestinal tract, bladder and kidney cortex (Powles, 1992a).

342

In a study of absorption, distribution and excretion, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material = 96.8%; radiochemical purity > 98%) as a single dose of 30 or 1000 mg/kg bw by gavage in saline or intravenously as a single dose at 30 mg/kg bw. A group of five male and five female rats was administered unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by [^{14}C]glyphosate as a single oral dose at 30 mg/kg bw. The animals were housed individually in metabolism cages from which urine, faeces and expired air were collected at regular intervals. The rats were terminated after 90% of the dose had been eliminated or 7 days after dosing, whichever was sooner. At necropsy, a blood sample was drawn and selected tissues removed.

Following administration of the single intravenous dose of 30 mg/kg, more than 84% of the radioactivity was eliminated in urine, mostly within 8 hours. Faecal elimination accounted for less than 3.5% of the administered radioactivity and only a very small proportion was eliminated in exhaled air; less than 1.4% remained in tissues and the residual carcass after termination. In contrast, faeces were the major route of elimination when [^{14}C]glyphosate was administered orally. Approximately 56–59% of the oral dose of 30 mg/kg was excreted in faeces, mostly within 12–36 hours. Urinary elimination of the oral dose was slower than for the intravenous dose, with 29–31% eliminated, mostly within 36 hours of dosing. Excretion was unaffected by administering unlabelled glyphosate for 14 days prior to dosing with [^{14}C]glyphosate, and the routes and rates of excretion of a high dose of [^{14}C]glyphosate (1000 mg/kg) were essentially identical to that of the low dose. There was no significant sex difference in the elimination of glyphosate for any dose regimen. Irrespective of the dose, route or frequency of duration, less than 1.4% of a dose was retained in tissues. The highest concentration of radioactivity was in bone and lower concentrations were in bone marrow, kidneys, liver, lungs and the residual carcass (Powles, 1992b).

In a study of absorption, distribution, excretion and metabolism, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material 98.9%; radiochemical purity > 98%) as a single dose at 10 or 600 mg/kg bw by gavage in water. For the excretion study, urine and faeces (5/sex) were collected at selected intervals for 168 hours. Animals were terminated at 168 hours post dosing and the radioactivity in blood and selected tissues analysed. For the plasma concentration study, blood samples (total nine per sex per dose) were drawn at selected intervals up to 168 hours. For the tissue distribution study, 12 rats (six male, six female) were administered single oral doses of either 10 or 600 mg/kg bw per day by gavage. The animals were divided into two groups of six (three per sex) and terminated by cervical dislocation 6 and 18 hours (the low-dose study) or 3 and 9 hours (for the high dose) after dosing, depending on the peak plasma concentrations and half the plasma concentration derived in the blood/plasma kinetics experiments. Samples of urine and faecal extracts from male and female rats were pooled and analysed directly by thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC).

During the 7-day observation period, up to about 23% and 30% of the radioactivity of the low dose was excreted in the urine of low- and high-dose animals, respectively. At both doses, about three quarters of the radioactivity was detected in the faeces within 7 days (75% for males and 84% for females, 10 mg/kg bw; 75% and 74%, 600 mg/kg bw; Table 4) (McEwen, 1995).

Table 4. Radioactivity in rat excreta and tissue over 168 hours after a single dose of ^{14}C-labelled glyphosate

	Percentage of administered radioactive dose (%)			
	10 mg/kg bw		600 mg/kg bw	
Excretion intervals (h)	Males	Females	Males	Females
Urine				
0–6	2.63	3.25	11.55	9.08

Excretion intervals (h)	Percentage of administered radioactive dose (%)			
	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
6–24	15.85	12.69	13.85	13.36
24–48	2.82	2.41	2.33	4.40
48–72	0.54	0.44	0.59	1.07
72–96	0.24	0.19	0.30	0.40
96–120	0.15	0.13	0.21	0.24
120–144	0.09	0.07	0.17	0.17
144–168	0.07	0.05	0.13	0.18
Cage wash	0.12	0.14	1.13	0.60
Subtotal (urine plus cage wash)	22.51	19.37	30.26	29.50
Faeces				
0–24	60.28	74.59	58.94	46.28
24–48	11.72	7.56	13.41	22.87
48–72	1.18	1.34	1.36	3.83
72–96	0.29	0.36	0.35	0.47
96–120	0.17	0.27	0.36	0.23
120–144	0.35	0.08	0.08	0.12
144–168	0.64	0.10	0.15	0.35
Subtotal faeces	74.63	84.30	74.65	74.15
Residual carcass	0.33	0.27	0.31	0.39
Total	97.47	103.94	105.22	104.04

bw: body weight

Source: McEwen (1995)

After a single dose of 10 mg/kg bw, peak mean concentrations of radioactivity in plasma occurred at 6 and 2 hours in males (0.22 µg eq/mL) and females (0.28 µg eq/mL) (Table 5). After a single oral dose of 600 mg/kg bw, peak mean concentrations of radioactivity in plasma occurred at 3 hours in both males (26 µg eq/mL) and females (29 µg eq/mL). The area under the concentration versus time–curve (AUC_t) was calculated at 400 and 355 µg eq/mL*hour in males and females, respectively. These values were around 120 times higher than the AUCt obtained in the low-dose group.

Table 5. Pharmacokinetic parameters of total rat plasma radioactivity following single oral doses of ^{14}C-labelled glyphosate

Parameter	Measures per administered dose			
	10 mg/kg bw		600 mg/kg bw	
	Males	Females	Males	Females
C_{max} (µg eq/mL)	0.2219	0.2789	25.97	28.84
T_{max} (hour)	6.00	2.00	3.00	3.00
AUC_t (µg eq/mL*hour)	3.20	3.70	399.90	355.30
AUC (µg eq/mL*hour)	3.80	4.20	419.00	–

Terminal rate constant (per hour)	0.0840	0.0887	0.1174	–[a]
Terminal half-life (hour)	8.30	7.80	5.90	–[a]
Absorption rate constant (per hour)	0.2963	0.4239	0.2845	0.4477

AUC: area under the plasma concentration–time curve; AUC_t: area under the curve calculated up to the last detectable sample (calculations done up to 24 hours); bw: body weight; C_{max}: maximum concentration; eq: equivalent; T_{max}: time to reach the maximum concentration

[a] Could not be calculated accurately as the values were at or close to the limit of reliable measurement.

Source: McEwen (1995)

There was no indication of accumulation of radioactivity in any tissue. Only the gastrointestinal tract, the stomach, muscles and the kidneys, the organs of excretion contained concentrations of radioactivity higher than the plasma (Table 6). High levels of radioactivity were detected in the content of stomach and the gastrointestinal tract. The radioactivity in most tissues had decreased to around the limit of detection 7 days after dosing.

Table 6. Radioactivity in male and female rat tissue over 168 hours after a single oral dose of 10 mg/kg bw ^{14}C-labelled glyphosate

	Proportion of administered dose over time (%)					
	Male[a]			Females[a]		
Tissue	**6 hours**	**18 hours**	**168 hours**	**6 hours**	**18 hours**	**168 hours**
Bone[b]	0.12	0.10	0.02	0.10	0.09	0.03
Carcass	2.00	2.69	0.33	1.69	3.03	0.27
Gastrointestinal tract	19.05	10.04	0.01	16.47	5.41	0.01
Gastrointestinal tract contents	31.56	4.89	0.01	34.54	14.30	0.01
Kidneys	0.79	0.36	< 0.01	0.67	0.26	< 0.01
Muscle (skeletal)	0.23	0.13	0.04	0.24	0.11	< 0.03
Stomach	3.47	0.60	0.60	2.56	0.62	< 0.01
Stomach contents	25.16	5.05	0.01	22.90	6.96	0.01
Plasma	0.12	0.03	< 0.01	0.13	0.03	< 0.01
Whole blood	0.20	0.04	< 0.03	0.15	0.05	< 0.03

bw: body weight

Results expressed as mean percentage (%) of applied dose, except bone, which is expressed as percentage (%) of applied dose/g

[a] $N = 5$

[b] $n = 3$

Source: McEwen (1995)

A major component of urine or the [14C]phosphonomethyl-labelled glyphosate-treated animals was unchanged glyphosate, accounting for 18–27% of both the administered doses. A minor component, accounting for 0.1–0.3% of the administered dose, was shown to co-chromatograph (using normal phase TLC and reverse phase HPLC) with aminomethylphosphonic acid.

Unchanged glyphosate was the major component of the faecal extract of the [^{14}C]phosphonomethyl-labelled glyphosate-treated animals, accounting for 65–78% of both the administered doses. Two minor metabolites accounted for 0.3–1.6% of the administered dose; one of these was shown to co-chromatograph with aminomethylphosphonic acid (McEwen, 1995).

345

In a series of experiments that compared the faecal and urinary excretion of ^{14}C-labelled glyphosate, five male and five female Alpk:APfSD rats were each given a single oral dose of 10 mg/kg bw and 1000 mg [^{14}C]phosphonomethyl-labelled glyphosate (radiochemical purity > 98%) in deionized water. Excretion was measured over 72 hours, after which the animals were terminated and the radioactivity in blood and selected tissues including residual carcasses analysed.

Excretion of radioactivity was rapid for both sexes and most of the administered dose was eliminated, principally in faeces, within 24 hours. Males excreted 13.0% and 88.5% of the lower dose and 16.7% and 89.6% of the higher dose in urine and faeces, respectively. Females excreted 10.6% and 88.7% of the lower dose and 17.5% and 84.5% of the higher dose in urine and faeces, respectively.

At termination, radioactivity in the tissues accounted for only 0.6% and 0.5% of the lower dose in males and females, respectfully. The highest concentrations were in bone (0.5 and 0.4 µg eq/g of the lower dose and 50 and 45 µg eq/g of the higher dose for males and females, respectively). All other tissue concentrations were 0.07 µg/g or less for the lower dose and 7 µg eq/g or less for the higher dose. No marked sex difference was seen in the tissue distribution of radioactivity (Davies, 1996a,b).

A similar experiment was conducted using five male and five female Alpk:APfSD rats pre-treated with 10 mg/kg bw of unlabelled glyphosate (purity 99.2%) for 14 days before being given the single oral dose of 10 mg/kg bw of [^{14}C]phosphonomethyl-labelled glyphosate (radiochemical purity > 98%) in deionized water. Once again, excretion was measured over 72 hours. The animals were then terminated and the radioactivity in blood and selected tissues including residual carcass analysed.

Excretion of radioactivity was rapid in both sexes and most of the administered dose was eliminated, principally in faeces, within 24 hours. Males excreted 10.6% and 86.6% and females 10.7% and 90.7% of the administered dose in urine and faeces, respectively.

At termination, tissue concentrations of radioactivity accounted for 0.5% of the administered dose in both sexes. The amount in the tissue and contents of the intestinal tract were 0.12% of the administered dose in both sexes. The highest concentrations were in bone (0.36 and 0.35 µg eq/g in males and females, respectively). All other tissue concentrations were 0.07 µg eq/g or lower. No marked sex difference was seen in the distribution of radioactivity in the tissues. Comparison of these results with those obtained when [^{14}C]glyphosate was administered without pretreatment shows that pre-dosing has no significant effect on either the routes or rates of elimination of a single dose of the radiolabelled test material (Davies, 1996c).

Two male and two female Alpk:APfSD rats were each given a single oral dose of 10 mg/kg bw [^{14}C]phosphonomethyl-labelled glyphosate (radiochemical purity > 96%) in deionized water. Excretion was measured throughout the study. At intervals of 24 and 48 hours after dosing, one rat of each sex was terminated and rapidly frozen for whole-body autoradiography.

Within 24 hours of dosing, male rats excreted 22.3% and 55.5% and female rats 11.9% and 83.8% of the administered dose in the urine and faeces, respectively. Within 48 hours of dosing, the remaining male rats excreted 34.0% and 60.5% and the female rats 12.5% and 91.2% of the administered dose in the urine and faeces, respectively.

The whole-body autoradiography showed no marked differences in the distribution of radioactivity between male and female rats. The high levels of radioactivity in the gastrointestinal tract were consistent with faeces being the predominant route of elimination; accordingly, these levels had declined markedly by 48 hours. The greatest intensity of tissue radiolabelling at both 24 and 48 hours was in bone. Some radioactivity was in the kidney after 24 hours but had declined by 48 hours. No significant levels of radioactivity were apparent in other tissues (Davies, 1996d).

In a study of absorption, distribution, excretion and metabolism, groups of five male and five female Sprague Dawley (Crl:CD BR) rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (two batches of unlabelled test material, purity 95.3% and 96.0%; radiochemical purity > 99%) as a single gavage dose of 1 or 100 mg/kg bw in water. For the excretion study, urine and faeces (5/sex per dose) were collected at selected times for 168 hours and samples pooled and analysed directly by TLC or HPLC. At 168 hours, the animals were terminated and radioactivity in blood and selected tissues analysed. For the pharmacokinetic study, blood was drawn (5/sex per dose) at selected intervals up to 72 hours after dosing. For the tissue distribution study, 12 male and 12 female rats were administered a single daily gavage dose of either 10 or 100 mg/kg bw. The treated animals were divided into four groups (three per sex) and terminated at 4, 12, 24 and 72 hours after dosing. For the biliary excretion study, seven male and seven female cannulated rats were administered a single gavage dose of 1 mg/kg bw. Urine, faeces and bile were collected periodically up to 48 hours after dosing.

Following a single gavage dose of 1 mg/kg bw, the major route of elimination was the faeces with 72.62% recovered in males and 62.40% in females, mostly within 24 hours of dosing (63.93% in males and 49.69% in females), suggesting this proportion of the dose was not systemically absorbed. During the 7-day observation period, 18.44% (male) and 27.15% (female) of radioactivity were recovered in the urine, representing the systemically absorbed dose. The remainder of the radioactivity was recovered in the cage wash (6.48% in males and 7.71% in females), cage debris (0.03% in males and 0.58% in females) and carcass (0.75% in males and 0.98% in females).

Following the single gavage dose of 100 mg/kg bw, elimination of radioactivity in the urine (39.42% in males and 43.07% in females) was quantitatively more significant than in to the low-dose group. Faecal elimination accounted for 41.23% in males and 42.37% in females. The remainder of the radioactivity was recovered in the cage wash (13.85% in males and 11.96% in females), cage debris (0.98% in male and 0.10% in female) and carcass (0.84% in male and 0.98% in female). Renal elimination was essentially complete in 48 hours.

In the cannulated rats dosed with 1 mg/kg bw by gavage, the majority of the administered dose was recovered in faeces (55.33% in male and 60.97% in female) in 48 hours. Renal elimination accounted for 27.45% in males and 24.21% in females. The remainder of the radioactivity was recovered in the cage wash (6.57% in male and 6.77% in female), cage debris (0.26% in male and 0.15% in female) and carcass (4.99% in male and 3.82% in female).

The mean terminal elimination half-lives were 10.86 hours and 8.07 hours with corresponding area under the plasma concentration–time curve (AUC) of 0.319 and 0.340 µg eq/mL*hour in males and females, respectively (Table 7). As the elimination half-lives could not be calculated for several high-dose animals, mean AUC_{0-24} (0.257 and 0.338 µg eq/mL*hour in males and females) were calculated to compare the results of both groups. Following a single oral dose of 100 mg/kg bw, mean AUC_{0-24} were 58.2 and 50.7 µg eq/mL*hour in males and females, respectively.

Table 7. Kinetic parameters in male and female rat plasma after a single oral dose of ^{14}C-labelled glyphosate

	Measures per administered dose			
	1 mg/kg bw		100 mg/kg bw	
Kinetic parameters	**Males**	**Females**	**Males**	**Females**
C_{max} (µg eq/mL)	0.016	0.037	8.909	7.634
T_{max} (hour)	3.900	8.000	3.600	4.000
AUC_{0-24} (µg eq/mL*hour)	0.257	0.338	58.200	50.700
AUC (µg eq/mL*hour)	0.319	0.340	–	–

341

Terminal half-life (hour)	10.860	8.065	–	–

AUC: area under the plasma concentration–time curve; AUC_{0-24}: area under the plasma concentration–time curve from time 0 to 24 hours; bw: body weight; C_{max}: maximum concentration; eq: equivalent; T_{max}: time to reach the maximum concentration

Source: Knowles & Mookherjee (1996)

At 1 mg/kg bw, radioactivity was detected in all tissues 4 hours post dose, indicating rapid absorption and distribution in the body. Apart from the gastrointestinal tract (and contents) and the carcass, the kidneys was the only tissue with any notable amounts throughout the observation period. At 72 hours, post-dose concentrations had decreased or plateaued to less than 2% of the administered dose in all tissues in both males and females, with the carcass containing most of the remaining radioactivity. At 100 mg/kg bw, all the tissues were exposed to radiolabelled material 4 hours post dose. Again, only the gastrointestinal tract, carcass and kidneys contained significant amounts of radioactivity. After 72 hours, concentrations had decreased or plateaued to less than 2% of the dose in all tissues in both sex, with the carcass containing most of the remaining radioactivity.

In conclusion, following oral administration of glyphosate at 1 mg/kg bw and 100 mg/kg bw, the absorption, distribution, metabolism and excretion was independent of dose level and sex. Metabolism of glyphosate was very low with more than 90% of the administered dose eliminated unchanged in the urine and faeces. Elimination was essentially completed by 48 hours, and the majority of the radioactivity was recovered in faeces (Knowles & Mookherjee, 1996).

Rabbits

In a pre-GLP study, glyphosate ^{14}C-labelled at the methylene carbon, at the C1-glycine carbon and at the C2-glycine carbon was dissolved in isotonic saline and administered by gavage to male New Zealand White rabbits fasted for 3 hours. In two replicate experiments, three rabbits were administered ^{14}C-methylene glyphosate, two were administered ^{14}C-C1-glycine glyphosate and two were administered ^{14}C-C2-glycine glyphosate. All the doses were within a range of 5.7–8.8 mg/kg bw.

Approximately, 80–97% of the oral dose of [^{14}C]glyphosate was excreted in the faeces and 7–11% in the urine over 120 hours. Less than 1% of the dose was exhaled. Approximately 1.2%, 0.7% and 0.1% of the dose was retained in the tissues (excluding gastrointestinal tract contents) for ^{14}C-C2-glycine, ^{14}C-C1-glycine and ^{14}C-methylene glyphosate, respectively. The radioactivity in the tissues differed between ^{14}C-C2-glycine and ^{14}C-C1-glycine by 4 or 5 times, but the ranking was similar: the liver had the highest concentrations followed by the kidney, the spleen, the heart, skeletal muscle and gonads, in that order. Only ^{14}C-C2-glycine radioactivity was incorporated in the fat (Colvin & Miller, 1973c).

(b) Intraperitoneal route

In the previously described Colvin & Miller (1973a) study, three treatment groups each with three male Wistar rats were dosed via intraperitoneal injection with ^{14}C-methylene glyphosate (2.33 mg/kg bw), ^{14}C-C1-glycine glyphosate (2.91 mg/kg bw) and ^{14}C-C2-glycine glyphosate (3.63 mg/kg bw). Within 12 hours, 74–78% of the ^{14}C-glyphosate was excreted in the urine. At 96 hours post-administration, total urinary excretion was 81–90% of the administered radioactivity and faecal excretion was 6–14% of the administered radioactivity, indicating that [^{14}C]glyphosate is also eliminated via the bile. The percentage of radioactivity recovered as expired $^{14}CO_2$ was slightly greater than that observed following oral administration (Section 1.1 (a)), but for all three radiolabels was less than 1% of the administered dose. Tissue retention was also greater than after oral administration, but was in all cases less than or equal or 1% of the administered dose (Colvin & Miller, 1973a).

[^{14}C]glyphosate with a radiochemical purity of 98% was administered by intraperitoneal injection to nine male and nine female Sprague Dawley rats at a dose level of 1150 mg/kg bw. The rats were subsequently housed in metabolism cages, and blood samples were collected from three to six rats at approximately 0.25, 0.50, 1, 2, 4, 6 and 10 hours. At approximately 0.5, 4 and 10 hours after dosing, three animals of each sex were terminated and the femoral bone marrow isolated. The plasma and bone marrow samples were analysed for radioactivity by liquid scintillation counting.

Peak levels of radioactivity were observed in plasma and bone marrow about 0.5 hours after dosing. When expressed as glyphosate acid equivalents, the peak values for bone marrow and plasma in males and females combined were approximately 340 and 1940 ppm, respectively. The radioactivity in plasma decreased rapidly but remained more constant in bone marrow over the 10 hours of the experiment. The analysis of the first-order elimination rates indicated a half-life of elimination from the plasma of approximately 1 hour for both males and females. Elimination from bone marrow was slower with a half-life of 4.2 hours for females and 7.6 hours for males (Ridley, 1983).

(c) Intravenous route

In a previously described study by Ridley & Mirly (1988) (Section 1.1 (a)), groups of male and female Sprague Dawley rats (Crl:CD(SD)BR) were injected with a single intravenous dose of 10 mg/kg bw [^{14}C]glyphosate into the lateral tail vein. Urine and faeces were collected at intervals for 7 days, and the animals were terminated and tissues and carcass analysed for radioactivity.

The majority of the dose – 79.0% in males and 74.5% in females – was excreted in the urine. Faecal excretion was 4.65% and 8.30% of the administered dose, respectively, which suggests that glyphosate is eliminated via the bile. Very little (< 0.1%) of the administered dose was found in the tissues and organs. An intravenous dose resulted in significantly higher levels of radioactivity in the residual carcasses than those found following oral dosing, with the highest concentrations in bone: 1.48 ppm glyphosate equivalents in males and 1.59 ppm glyphosate equivalents in females (Ridley & Mirly, 1988).

In the previously described absorption, distribution and excretion study by Powles (1992b) (section 1.1(a)), groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material, 96.8%; radiochemical purity > 98%) as a single dose of 30 or 1000 mg/kg bw by gavage in saline or intravenously as a single dose at 30 mg/kg bw.

Following administration of the 30 mg/kg bw intravenous dose, more than 84% of the radioactivity was eliminated in urine, mostly within 8 hours. Faecal elimination accounted for less than 3.5% of the administered radioactivity. Only a very small proportion of the radioactivity was eliminated in exhaled air and less than 1.4% remained in the tissues and the residual carcass after the animals were terminated. In contrast, faeces were the major route of elimination when [^{14}C]glyphosate was given orally (Powles, 1992b).

(d) Intramuscular route

In a two-phase excretion study, [^{14}C]glyphosate was mixed with isopropylamine and unlabelled glyphosate isopropylamine salt and dissolved in water to make a solution of 4 mg glyphosate/mL. One millilitre of this solution was injected into the thigh muscle of each of four male Rhesus monkeys. Urine samples were collected at intervals for up to 7 days.

During the 7-day collection period following intramuscular injection, 89.9% of the applied radioactivity was excreted in urine. The overall urinary elimination half-life was 19.7 hours. There were two distinct phases to the elimination kinetics, a rapid phase with a half-life of 6.9 hours (over the first 24 hours) and a slow phase with a half-life of 35.1 hours (Maibach, 1983).

GLYPHOSATE 89–296 JMPR 2016

(e) Dermal route

In vitro

The absorption of glyphosate acid (purity 95.93%) from a dried glyphosate wet cake preparation through abraded rabbit whole skin was measured in vitro over 24 hours. The dose was placed on the abraded skin at a nominal rate of 79.8 mg/cm^2 (48.3 mg glyphosate acid/cm^2), calculated as equivalent to the 5000 mg/kg bw per day dose administered to rabbits in an in vivo dermal toxicity study (Johnson, 1982). The diffusion cell was left unoccluded for 6 hours, and the surface of the skin was then decontaminated with a sponge wash. Physiological saline was used as the receptor fluid.

The total recovery of the individual cells was 87.3–98.2%, with an overall mean recovery of 93.3% of applied dose. The majority of the applied glyphosate acid (mean 87.9%) was washed off the skin at 6 hours, with a further 2.38% washed off at 24 hours. A small proportion (0.041%) of the dose applied was recovered from the epidermis, with 0.243% remaining in the dermis. The mean amount of glyphosate acid that penetrated abraded rabbit skin into the receptor fluid over the entire 24-hour experimental period was 1177 µg/cm^2, corresponding to 2.42% of the applied dose. The reported total potentially absorbable amount, represented by the mean absorbed dose together with the mean amount in the remaining dermis, was 2.66%. The results of this in vitro study indicated that dermal absorption of glyphosate through abraded rabbit skin is slow (Hadfield, 2012a).

The penetration through human epidermis of glyphosate from a formulation concentrate was measured in vitro over 24 hours. The glyphosate formulation concentrate, containing a nominal 360 g/L of an isopropylamine salt of glyphosate at a 1:133 weight per volume (w/v) aqueous dilution was applied to the epidermal membranes at a rate of 10 µL/cm^2 and left unoccluded for 8 hours.

Penetration of glyphosate was fastest between 0 and 2 hours after application (0.914 µg/cm^2 per hour). The mean penetration rate slowed to 0.074 µg/cm^2 per hour between 2 and 24 hours. The mean amount penetrated over the entire 24-hour exposure period was 3.51 µg/cm^2, corresponding to 0.096% of the applied dose (Hadfield, 2012b).

The absorption and distribution of glyphosate from a 360 g/L soluble (liquid) concentrate (MON 79545) through human epidermis was measured in vitro. The doses were applied as the concentrate formulation (450 g/L of glyphosate) and as 1:15.6 volume per volume (v/v) and 1:188 v/v (nominally 28.8 and 2.4 g/L of glyphosate) aqueous spray dilutions of the formulation. ^{14}C-radiolabelled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to the epidermal membranes at a rate of 10 µL/cm^2 and left unoccluded for 24 hours.

The mean total amount of absorbed glyphosate in 24 hours was 0.573 µg/cm^2 (0.012% of applied dose) from the 450 g/L concentrate formulation. From the 1:15.6 v/v and 1:188 v/v aqueous dilutions, the mean total amounts of absorbed glyphosate in 24 hours were 0.379 and 0.021 µg/cm^2 (0.129% and 0.082% of applied dose), respectively (Ward, 2010a).

The absorption and distribution of glyphosate from a 360 g/L soluble (liquid) concentrate (MON 79351) through human epidermis was measured in vitro when doses were applied as the concentrate formulation (480 g/L of glyphosate) and as 1:16.7 v/v and 1:200 v/v (nominally 28.7 and 2.4 g/L) aqueous spray dilutions of the formulation. ^{14}C-radiolabelled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The doses were applied to the epidermal membranes at a rate of 10 µL/cm^2 and left unoccluded for 24 hours.

The mean total amount of absorbed glyphosate in 24 hours was 0.342 µg/cm^2 (0.0070% of applied dose) from the 480 g/L concentrate formulation. From the 1:16.7 v/v and 1:200 v/v aqueous

dilutions of the formulation, the mean total amounts of absorbed glyphosate in 24 hours were 0.0.553 and 0.015 µg/cm^2 (0.182% and 0.0488% of applied dose), respectively (Ward, 2010b).

The absorption and distribution of glyphosate from a 360 g/L soluble (liquid) concentrate was measured in vitro through human epidermis when it was applied as the concentrate formulation (360 g/L of glyphosate) and a 3:200 v/v aqueous spray strength dilution of the formulation. ^{14}C-radiolabelled glyphosate was incorporated into the concentrate formulation and dilutions prior to application. The actual concentrations achieved were 364 g/L and 6.70 g/L of glyphosate for the concentrate and the spray dilution, respectively. The doses were applied to the epidermal membranes at a rate of 5 µL/cm^2 and left unoccluded for 24 hours.

For the concentrate, the mean rate of absorption in 24 hours was 0.02 µg/cm^2 per hour. For the 3:200 v/v aqueous dilution, the mean rate of absorption in 24 hours was 0.001 µg/cm^2 per hour. For the concentrate, mild skin washing at 6 and 24 hours removed practically all of the applied dose from the surface of epidermal membrane. For the 3:200 v/v spray dilution skin washing at 6 and 24 hours removed 90.8% and 87.9% of the applied dose, respectively (Davies, 2003).

In vivo

In the dermal penetration phase of the Maibach (1983) study described above (section 1.1 (d)), 25 µL of [^{14}C]glyphosate solution containing 8.9 mg glyphosate was placed on the shaved abdomens (7.9 cm^2 area) of six male Rhesus monkeys. After 24 hours, each abdomen was swabbed twice with water, twice with acetone and again twice with water to remove any residual glyphosate. Urine samples were collected periodically for up to 7 days post application.

The washing procedure removed 14.2% of the applied ^{14}C label. A mean total of 1.8% of the applied dose of [^{14}C]glyphosate was recovered in the urine during the 7-day collection period. Glyphosate penetrated the monkey skin slowly as only 0.4% of the topically applied dose appeared in the urine after 24 hours. The urinary elimination half-life for topically applied glyphosate was 59 hours (Maibach 1983).

1.2 Biotransformation

Seven test groups, each with an equal number (between three and five) of male and female Sprague Dawley Crl:CD(SD)BR rats, were dosed with *N*-(phosphono[^{14}C]methyl)glycine glyphosate. The radiochemical purity was 98% or greater. Single oral doses were administered by gastric intubation whereas the intravenous doses were injected into the lateral tail vein. Comparison of the areas under the curves for radioactivity levels in whole blood after oral (mean dose for males: 10.2 mg/kg bw; for females: 10.6 mg/kg bw) and intravenous (mean dose for males: 10.7 mg/kg bw; for females: 11.0 mg/kg bw) administration of radiolabelled glyphosate indicated that absorption of the oral dose of glyphosate at the 10 mg/kg bw dose level was 30.4% for males and 35.4% for the females. Glyphosate was isolated as the predominant radioactive fraction in urine (overall recovery of 81.3%) and faeces (overall recovery of 99.2%), and was positively identified in each case by various analytical methods. The minimum glyphosate content as a per cent of either urine or faecal extract contained radioactivity in all of the individual rat excreta samples at 97.46%. HPLC analyses further indicated that glyphosate in the excreta accounted for 98.50–99.33% of the administered [^{14}C]glyphosate.

In groups orally treated with a mean dose of 9.41 mg/kg bw for males and 9.28 mg/kg bw for females and with a mean dose of 10.7 mg/kg bw for males and 10.3 mg/kg bw for females, there was evidence that glyphosate was metabolized to produce 0.2–0.3% and 0.4% AMPA, respectively. The remainder of the radioactivity in the excreta was due to low-level impurities in the dosing material or changes during storage of the excreta samples (Howe, Chott & McClanahan, 1988).

351

Urine and faeces samples from the previously described study by Powles (1992b) (Section 1.1 (c)) were analysed for identification of glyphosate metabolites. Briefly, groups of five male and five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material: 96.8%; radiochemical purity > 98%) as a single dose of 30 or 1000 mg/kg bw by gavage in saline or intravenously as a single dose at 30 mg/kg bw. Another group of five male and five female rats were administered unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 30 mg/kg bw.

The recovery of radioactivity from urine and faecal samples was generally greater than 90%. For both dose groups only one major region of radioactivity was detected when extracts were analysed by either liquid chromatography or TLC and this co-chromatographed with a glyphosate standard. The identity of the major component as glyphosate was confirmed by comparing its Fourier transform infrared spectroscopy spectrum with a glyphosate standard. Small amounts of other components were detected but no radiolabelled metabolites were identified (Powles, 1992b).

Urine and faeces samples from the previously described McEwen (1995) study (section 1.1 (a)) were analysed for identification of glyphosate metabolites. Briefly, groups of five female Sprague Dawley rats were administered [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test material: 98.9%; radiochemical purity > 98%) as a single dose at 10 or 600 mg/kg bw by gavage in water. Urine and faeces were collected for 7 days and analysed for metabolites.

The major urinary component was unchanged glyphosate, accounting for 18–27% of the administered dose. Only 0.1–0.3% of the administered dose was shown to co-chromatograph, using normal phase TLC and reverse phase HPLC, to aminomethylphosphonic acid. Faecal extract contained 65–78% of administered dose as unchanged glyphosate. Two minor metabolites were in faecal extract, accounting for 0.3–1.6% of the administered dose; one of these two metabolites was shown to co-chromatograph with aminomethylphosphonic acid (McEwen, 1995).

The biotransformation of ^{14}C-labelled glyphosate was investigated in male and female rats administered either as a single 10 mg/kg dose or a single 10 mg/kg dose following repeated oral doses of 10 mg/kg unlabelled glyphosate or as a single 1000 mg/kg bw dose. The metabolites in excreta from the Davies (1996a,b,c) studies were identified (Section 1.1 (a)). In addition, a single oral dose of 1000 mg/kg of [^{14}C]glyphosate (97.8 radiochemical purity) was administered to male and female Alpk:AP$_f$SD rats fitted with a bile duct cannulae. The structural identification of metabolites isolated from urine, bile and faeces, collected over 48 hours (biliary study) or 72 hours, was characterized using various analytical methods.

Biliary excretion of radioactivity over 48 hours was negligible, 0.055% and 0.062% of the administered dose for male and female rats, respectively. The greater percentage of excreted dose was in faeces in both male (39.1%) and female rats (30.5%). Urinary excretion accounted for 20.8% of the administered dose in male rats and 16.3% of the administered dose in female rats. In cannulated rats, the excreted radioactivity (including cage wash) after 48 hours accounted for 62.5% and 52.0% of the administered dose in male and female rats, respectively.

The main urinary metabolite was unchanged glyphosate, which accounted for virtually the entire radioactivity present, with minor amounts of AMPA, which represented less than 1% of the dose in each study (see Table 8). Solvent extraction of faeces, collected from the various excretion and tissue distribution studies, resulted in the extraction of 53–79% of the radioactivity present. In each case the extracts contained a single peak, which corresponded to unchanged glyphosate (Macpherson, 1996).

352

Table 8. Quantification of glyphosate metabolites as percentages of single doses of ^{14}C-labelled glyphosate administered orally to rats

		Percentage of administered dose (%)					
		Low-dose study 10 mg/kg bw		Repeat dose study[a] 10 mg/kg bw		High-dose study 1000 mg/kg bw	
Sample	Analyte	Male	Female	Male	Female	Male	Female
Urine	Glyphosate	12.7	10.5	10.5	10.5	16.0	16.7
	AMPA	0.2	0.1	< 0.1	< 0.1	0.6	0.7
Faeces	Glyphosate	74.8	55.2	52.9	72.1	79.3	63.9
Total	Glyphosate	87.5	65.7	63.3	82.6	95.3	80.6
	AMPA	0.2	0.1	< 0.1	< 0.1	0.6	0.7

AMPA: aminomethylphosphonic acid; bw: body weight

[a] Following 14 repeated oral doses of 10 mg/kg bw unlabelled glyphosate.

Source: Macpherson, 1996

Urine and faeces samples from the previously described Knowles & Mookherjee (1996) study (Section 1.1 (a)) were analysed for identification of glyphosate metabolites. Briefly, five female Sprague Dawley (Crl:CD BR) rats were administered [^{14}C]phosphonomethyl-labelled glyphosate as a single dose at 1 or 100 mg/kg bw by gavage in water. For the excretion study, urine and faeces (5/sex per dose) were collected at selected times for 168 hours.

Metabolite profiles of pooled urine and faecal samples were investigated by HPLC. Only one major peak was detected in urine and faeces (> 90% of the total activity); this was subsequently identified as glyphosate. A minor component observed in the radiochromatograms had a similar retention time to AMPA; however, it could not be positively identified due to very low levels (Knowles & Mookherjee, 1996).

2. Toxicological studies

2.1 Acute toxicity

The results of acute toxicity studies of glyphosate (including skin and eye irritation and dermal sensitization studies) are summarized in Table 9.

Table 9. Summary of acute toxicity studies with glyphosate

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Oral					
Mouse	ICR	M + F	96.7	> 10 000	Shirasu & Takahashi (1975)
Mouse	NMRI	M + F	98.6	> 2 000	Dideriksen (1991)
Mouse	ICR(Crj:CD-1)	M + F	95.68	> 5 000 (M) > 5 000 (F) > 5 000 (combined)	Komura (1995a)
Mouse	ICR(Crj:CD-1)	M + F	62.34% glyphosate isopropylamine salt	> 5 000	Enami & Nakamura (1995)
Rat	Sprague Dawley	F	96.40 & 96.71	> 5 000	Komura (1995b)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rat	HanRcc: WIST	F	96.66	> 2 000	Simon (2009a)
Rat	CD/Crl:CD(SD)	F	97.52	> 2 000	Haferkorn (2009a)
Rat	Sprague Dawley	F	96.40 & 96.71	> 5 000	You (2009a)
Rat	CD/Crl:CD(SD)	F	95.23	> 2 000	Haferkorn (2010a)
Rat	CD/Crl:CD(SD)	F	97.3	> 2 000	Haferkorn (2010b)
Rat	Sprague Dawley derived	F	97.23	> 5 000	Merkel (2005a)
Rat	Wistar Hannover	F	98.05	> 2 000	Do Amaral Guimaraes (2008a), with addendum dated 2010
Rat	HanRcc: WIST(SPF)	F	95.1	> 2 000	Talvioja (2007a)
Rat	Sprague Dawley	M + F	97.76	> 5 000 (M) > 5 000 (F) > 5 000 (combined)	Reagan & Laveglia (1988a)
Rat	Wistar	M + F	99	5 600 (combined)	Heenehan, Rinehart & Braun (1979)
Rat	Sprague Dawley	M + F	85.5	> 5 000	Blaszcak (1988a)
Rat	Sprague Dawley	M + F	98.6	> 5 000	Cuthbert & Jackson (1989a)
Rat	Alpk:APfSD (Wistar derived)	M + F	95.6	> 5 000 (male) > 5 000 (female) > 5 000 (combined)	Doyle (1996a)
Rat	HanRcc:WIST(SPF)	F	96.1	> 5 000	Arcelin (2007a)
Rat	RjHan:WI	F	96.3	> 5 000	Tavaszi (2011a)
Rat	Wistar	M + F	99	5 600	Heenehan (1979a)
Rat	Sprague Dawley derived	M + F	62% glyphosate isopropylamine salt	> 5 000	Moore (1999)
Acute dermal					
Rat	Sprague Dawley	M + F	Not reported	> 2 000	Cuthbert & Jackson (1989b)
Rat	Sprague Dawley	M + F	96.40 & 96.71	> 5 050	You (2009b)
Rat	SD(Crj:CD)	M + F	95.68	> 2 000	Komura (1995c)
Rat	HanRcc: WIST(SPF)	M + F	96.66	> 2 000	Simon (2009b)
Rat	CD/Crl:CD(SD)	M + F	97.52	> 2 000	Haferkorn (2009b)
Rat	CD/Crl:CD(SD)	M + F	95.23	> 2 000	Haferkorn (2010c)
Rat	CD/Crl:CD(SD)	M + F	96.6	> 2 000	Haferkorn (2010d)
Rat	Sprague Dawley	M + F	97.23	> 5 000	Merkel (2005b)
Rat	Wistar Hannover	M + F	98.05	> 2 000	Do Amaral Guimaraes (2008b)
Rat	HanRcc: WIST(SPF)	M + F	95.1	> 2 000	Talvioja (2007b)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rat	Alpk:APfSD (Wistar derived)	M + F	95.6	> 2 000	Doyle (1996b)
Rat	HanRcc: WIST(SPF)	M + F	96.1	> 5 000	Arcelin (2007b)
Rat	RjHan (WI) Wistar	M + F	96.3	> 5 000	Zelenak (2011a)
Rabbit	New Zealand White	M + F	85.5	> 5 000	Blaszcak (1988b)
Rabbit	New Zealand White	M + F	97.76	> 5 000	Reagan (1988a)
Rabbit	New Zealand White	M + F	99	> 5 000	Heenehan (1979b)
Inhalation (nose only)					
Rat	CD/Crl:CD(SD)	M + F	96.6	> 5.18	Haferkorn (2010e)
Rat	F344/DuCrj(SPF)	M + F	97.56	> 5.48	Koichi (1995)
Rat	HsdRcc Han	M + F	96.66	> 5.04	Griffiths (2009)
Rat	CD/Crl:CD(SD)	M + F	97.52	> 5.12	Haferkorn (2009c)
Rat	CD/Crl:CD(SD)	M + F	95.23	> 5.02	Haferkorn (2010f)
Rat	Sprague Dawley	M + F	96.40 & 96.71	> 2.24	Carter (2009)
Rat	Sprague Dawley	M + F	97.23	> 2.04	Merkel (2005c)
Rat	Not reported	M + F	98.05	> 5.21	Dallago (2008)
Rat	HanRcc: WIST(SPF)	M + F	95.1	> 3.252	Decker (2007)
Rat	Alpk:APfSD (Wistar derived)	M + F	95.6	> 4.43	Rattray (1996)
Rat	Wistar RjHan (WI)	M + F	96.9	> 5.04	Nagy (2011)
Rat	Sprague Dawley	M + F	62% glyphosate isopropylamine	> 2.08	Wnorowski (1999)
Rat	Hsd:Sprague Dawley	M + F	47.2% glyphosate acid equivalent	> 5.27	Bonnette (2004)
Primary dermal irritation					
Rabbit	New Zealand White	M + F	95.1	Non-irritating	Talvioja (2007c)
Rabbit	Himalayan	M	95.23	Non-irritating	Leuschner (2009a)
Rabbit	New Zealand White	F	97.56	Non-irritating	Hideo (1995a)
Rabbit	Himalayan	M	97.52	Non-irritating	Leuschner (2009c)
Rabbit	Himalayan	M	96.6	Non-irritating	Leuschner (2010a)
Rabbit	New Zealand White	M + F	96.71	Non-irritating	You (2009c)
Rabbit	New Zealand White	M	97.23	Non-irritating	Merkel (2005d)
Rabbit	New Zealand White	F	98.05	Non-irritating	Canabrava Frossard de Faria (2008a)
Rabbit	New Zealand White	M + F	97.76	Non-irritating	Reagan & Laveglia (1988b)
Rabbit	New Zealand White	M + F	99	Slightly irritating	Heenehan (1979c)
Rabbit	New Zealand White	F	95.6	Non-irritating	Doyle (1996c)

Species	Strain	Sex	Purity (%)	LD_{50} (mg/kg bw) / Result	Reference
Rabbit	New Zealand White	M + F	96.1	Non-irritating	Arcelin (2007c)
Rabbit	New Zealand White	M	96.3	Mildly irritating	Zelenak (2011b)
Rabbit	New Zealand White	M + F	85.5	Slightly irritating	Blaszcak (1988c)
Eye irritation					
Rabbit	New Zealand White	M + F	95.1	Mildly irritating	Talvioja (2007d)
Rabbit	Himalayan	M	95.23	Moderately irritating	Leuschner (2009b)
Rabbit	New Zealand White	F	97.56	Severely irritating	Hideo (1995b)
None	n/a	–	Not stated	pH of a 1% solution in water was 1.93. Not tested because pH < 2 indicates corrosive properties	Simon (2009c) [a]
Rabbit	Himalayan	M	97.52	Mildly irritating	Leuschner (2009d)
Rabbit	Himalayan	M	96.6	Mildly irritating	Leuschner (2010b)
Rabbit	New Zealand White	M + F	96.40 & 96.71	Moderately irritating	You (2009d)
Rabbit	New Zealand White	M	97.23	Moderately irritating	Merkel (2005e)
Rabbit	New Zealand White	M + F	98.05	Severely irritating	Canabrava Frossard de Faria (2008b)
Rabbit	New Zealand White	Not reported	97.76	Severely irritating	Reagan & Laveglia (1988c)
Rabbit	New Zealand White	F	95.6	Mildly irritating	Johnson (1997)
Rabbit	New Zealand White	M + F	96.1	Mildly irritating	Arcelin (2007d)
Rabbit	New Zealand White	M	96.3	Severely irritating	Tavaszi (2011b)
Rabbit	New Zealand White	M + F	85.5	Moderately irritating	Blaszcak (1988d)
Rabbit	New Zealand White	M + F	46.6	Non-irritating	Blaszcak (1998c)
Rabbit	New Zealand White	M + F	57.8% glyphosate potassium (47.13% glyphosate acid equivalent)	Mildly irritating	Bonnette (2001)
Rabbit	New Zealand White	M + F	Not reported (MON 0139)	Non-irritating	Branch (1981)
Rabbit	New Zealand White	Not specified	90.8% (MON 8722)	Mildly irritating.	Busch (1987a)
Rabbit	New Zealand White	Not specified	70.7% (MON 8750)	Mildly irritating	Busch (1987b)
Rabbit	New Zealand White	Not specified	99	Moderately irritating	Heenehan (1979d)
Rabbit	New Zealand White	Not specified	97.76	Severely irritating	Reagan (1988b)

F: female; LD_{50}: median lethal dose; M: male

[a] According to Simon (2009c): "A 1% w/w solution of glyphosate technical in purified water was found to have a pH of 1.93. According to Council Regulation (EC) No. 440/2008, B.5. and OECD Guidelines 405, a test item is not required to be tested if the pH value is less than 2, because it is assumed that the test item has corrosive properties... Therefore, no eye irritation with glyphosate technical will be performed"

356

(a) Oral toxicity

Mice

Groups of 10 ICR mice of each sex were administered a single dose of glyphosate (purity 96.7%) at 1000, 5000 or 10 000 mg/kg bw orally by gavage and were observed for 14 days before termination.

Decreased locomotor activity was observed in all the mice at doses of 5000 mg/kg bw and higher. Two of high-dose males and one of the high-dose females died; the others recovered fully within 2 days. No abnormalities were found during necropsy.

The acute oral median lethal dose (LD_{50}) of glyphosate (96.7%) in mice was greater than 10 000 mg/kg bw (Shirasu & Takahashi, 1975).

Groups of five male and five female Bom:NMRI mice were administered a single dose of glyphosate (purity 98.6%) at 2000 mg/kg bw by gavage.

All the animals survived until the scheduled termination (day 14). Toxicological signs included piloerection and sedation in all mice on day 1. No macroscopic abnormalities were observed at necropsy.

The acute oral LD_{50} of glyphosate (98.6%) in mice was over 2000 mg/kg bw (Didriksen, 1991).

Five male and five female ICR(Crj:CD-1) mice were orally dosed with 5000 mg/kg bw glyphosate (purity 95.68%). The test material was administered as a 25% suspension in 0.5% carboxymethylcellulose (CMC) sodium solution at 20 mL/kg bw.

Signs of toxicity observed at 1 and/or 3 hours after administration included decreased spontaneous activity in one female and one male; another male was sedate and had a hunched posture. One male lost a slight amount of weight on days 0–7 after dosing, but all the mice gained weight over the 14-day observation period. There were no observed abnormalities at necropsy.

The acute oral LD_{50} of technical (95.68%) glyphosate in mice was greater than 5000 mg/kg bw (Komura, 1995a).

Five male and five female ICR(Crj:CD-1) mice were dosed with a formulation (described as a light viscous solution with a specific gravity of 1.23) containing 62.34% glyphosate isopropylamine salt. The test material was administered undiluted.

None of the mice died and there were no signs of toxicity. There was a slight retardation in mean body-weight gain in the males from day 0–7 compared with their controls (5000 mg/kg bw: 32.8–35.1 g; controls: 32.6–37.3 g). No gross pathological abnormalities were observed at gross necropsy.

The mouse acute oral LD_{50} of a formulation containing 62.34% glyphosate isopropylamine salt was greater than 5000 mg/kg bw (Enami & Nakamura, 1995).

Rats

In an acute oral toxicity study, five male and five female Sprague Dawley (Crj:CD) rats were orally dosed with 5000 mg/kg bw glyphosate (purity 95.68%). The test material was administered as a 25% suspension in 0.5% CMC sodium solution at 20 mL/kg bw.

There were no mortalities, but spontaneous motor activity was decreased in five male and three females, and one male had salivation. All the rats gained weight on days 0–7 and 7–14 after dosing. No abnormalities were seen at necropsy.

The acute oral LD_{50} of technical (95.68%) glyphosate in male and female rats was greater than 5000 mg/kg bw (Komura, 1995b).

Three female albino Sprague Dawley rats were administered 5000 mg/kg bw glyphosate (purity, 96.40% and 96.71%) by gavage. The test material was mixed with deionized water and administered as a 40% suspension at 12.5 mL/kg bw.

There were no mortalities. One rat showed slight to moderate signs of salivation, piloerection, diarrhoea, polyuria and decrease in activity, with recovery by day 8. The other two rats showed no indications of toxicity. All the rats gained weight days on days 0–7 and 7–14 after dosing. There were no observed abnormalities at necropsy.

The acute oral LD_{50} of technical (96.40% and 96.71%) glyphosate in female rats was greater than 5000 mg/kg bw (You, 2009a).

Two groups of three female HanRcc:WIST rats were orally dosed with 2000 mg/kg bw technical glyphosate (purity 96.66%). The test material was administered as a 20% suspension in purified water at a dose volume of 10 mL/kg.

All the rats survived. There were no signs of toxicity. Body-weight gain was normal and no macroscopic lesions were observed at necropsy.

The acute oral LD_{50} of technical (96.66%) glyphosate in rats was greater than 2000 mg/kg bw (Simon, 2009a).

Two groups of three female CD/Crl:CD(SD) rats were orally dosed with 2000 mg/kg bw technical glyphosate at purities of 97.52%, 95.23% and 97.3%. The test material was administered as a 20% suspension in 0.8% aqueous hydroxypropylmethylcellulose gel at a dose volume of 10 mL/kg.

All the rats survived. There were no signs of toxicity in the case of any of the test material purity. Body-weight gain was normal, and no pathological findings were noted at necropsy.

The acute oral LD_{50} of technical glyphosate (97.52%, 95.23% and 97.3%) in female rats was greater than 2000 mg/kg bw (Haferkorn, 2009a, 2010a,b).

Three female Sprague Dawley–derived albino rats were orally dosed with 5000 mg/kg bw technical glyphosate (purity 97.23%). The test material was administered as a 50% w/v suspension in distilled water (specific gravity: 1.252 g/mL).

All the rats survived. Clinical signs exhibited by all the rats were diarrhoea, anogenital and facial staining and/or reduced faecal volume, with recovery by day 4. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no gross abnormalities at necropsy. The acute oral LD_{50} of technical (97.23%) glyphosate in female rats was greater than 5000 mg/kg bw (Merkel, 2005a).

Two groups of three female Wistar Hannover rats were orally dosed with 2000 mg/kg bw technical glyphosate (purity 98.05%). The test material was mixed with deionized water to form a dosing mixture containing 200 mg/mL glyphosate technical.

All the rats survived. There were no signs of toxicity, all gained weight on days 0–7 and 7–14 after dosing, and there were no specific signs at necropsy.

358

The acute oral LD_{50} of technical (98.05%) glyphosate in female rats was greater than 2000 mg/kg bw (Do Amaral Guimaraes, 2008a, with an addendum dated 2010).

Two groups of three female HanRcc:WIST(SPF) rats were administrated 2000 mg/kg bw technical glyphosate (purity 95.1%) by gavage. The test material was diluted in polyethylene glycol (PEG 300) to 0.2 g/mL and administered at a dosing volume of 10 mL/kg.

All the rats survived. All showed piloerection at 1–3 or 2–3 hours after dosing. No other clinical signs were observed. All gained weight on days 1–8 and 8–15 after dosing. There were no macroscopic signs at necropsy.

The acute oral LD_{50} of technical (95.1%) glyphosate in female rats was greater than 2000 mg/kg bw (Talvioja, 2007a).

Five male and five female Sprague Dawley rats were orally dosed with 5000 mg/kg bw of technical glyphosate (purity 97.76%). The test material was administered as a 50% w/v aqueous suspension.

All the rats survived. All had diarrhoea, with recovery by day 4. In addition, three of the male and two of the female rats had wet abdomens ("apparent urinary incontinence") and one male and one female had hair loss on the abdomen at termination. All gained weight on days 1–8 and 8–15 after dosing. No internal abnormalities were observed at necropsy.

The acute oral LD_{50} of technical (97.76%) glyphosate in rats was greater than 5000 mg/kg bw (Reagan & Laveglia, 1988a).

Groups of five male and five female Wistar albino rats were dosed with 2.5, 3.5 5.0, 7.0 or 9.9 g/kg of technical glyphosate (purity 99%) administered as a 25% solution) in distilled water.

At 2.5 g/kg one of the five males died; at 3.5 g/kg one of the males died; at 5.0 g/kg three females died; at 7.0 g/kg all the males and three females died; at 9.9 g/kg all the animals died. Signs of toxicity included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy and faecal staining of the abdomen. The rats that died at 2.5 g/kg (day 5) and 3.5 g/kg (day 8) had considerable weight loss. At 7 and 9.9 g/kg, all the deaths occurred on day 1, except for one 9.9 g/kg male, which died on day 12. At necropsy, the male that died on day 5 after dosing at 2.5 g/kg had urinary and faecal staining of the abdomen, bright red lungs, stomach containing dark red fluid, upper intestines containing dark grey fluid, lower intestines distended with air and containing yellow fluid. The male that died on day 8 after dosing at 3.5 g/kg had white lungs. Almost all the surviving rats at 2.5 and 3.5 g/kg had red spots on the lungs, and mottled or purple livers. Surprisingly, most of the surviving rats at 5.0 g/kg had no visible abnormalities.

The oral LD_{50} (combined sexes) of technical glyphosate in rats was calculated to be 5.6 g/kg (95% confidence limits: 4.9–6.3 g/kg) (Heenehan, Rinehart & Braun, 1979).

Groups of five male and five female fasted CD Sprague Dawley–derived rats were administered glyphosate (purity 85.5%) as a single dose at 5000 mg/kg bw orally by gavage and observed for 14 days before termination.

All the animals survived until termination. One of the females exhibited weight loss on day 7 after dosing but gained weight on days 7–14. Toxicological signs included wet rales, faecal staining, urinary staining and soft stool. Some animals had decreased feed consumption after dosing, which continued in one animal through day 2. No gross abnormalities were found at necropsy (day 14).

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw (Blaszcak, 1988a).

Groups of five male and five female fasted Sprague Dawley rats were administered a single dose of glyphosate (purity 98.6%) at 5000 mg/kg bw orally by gavage and observed for 14 days before termination.

All the rats survived until termination. Toxicological signs included piloerection, reduced activity and ataxia through day 9. No gross abnormalities were found during necropsy.

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw (Cuthbert & Jackson, 1989a).

Five male and five female Alpk:APfSD (Wistar-derived) rats were dosed at 5000 mg/kg bw with technical glyphosate (purity 95.6%) administered as a 0.5 g/mL suspension in deionized water.

None of the rats died and there were no signs of toxicity. All gained weight days 1–8 and 8–15 after dosing. At necropsy, two of the males and two of the females had mottled or red areas on the lungs and one male had red areas on the thymus.

The acute oral LD_{50} of technical (95.6%) glyphosate in rats was greater than 5000 mg/kg bw (Doyle, 1996a).

Three female HanRcc:WIST(SPF) rats were dosed at 5000 mg/kg bw with technical glyphosate (purity 96.1%) administered as a 0.5 g/mL suspension in purified water.

None of the rats died. All had slightly ruffled fur (persisting in one rat through day 3) and all had hunched posture from 1–5 or 2–5 hours after dosing. All gained weight on days 1–8 and 8–15 after dosing. There were no macroscopic findings at gross necropsy.

The acute oral LD_{50} of technical (purity 96.1%) glyphosate in female rats was greater than 5000 mg/kg bw (Arcelin, 2007a).

Three female RjHan:WI rats were dosed at 5000 mg/kg bw with technical glyphosate (purity 96.3%), administered as a 0.5 g/mL suspension in 0.5% CMC.

None died and there were no signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. At necropsy, no abnormalities were noted.

The acute oral LD_{50} in female rats was greater than 5000 mg/kg bw (Tavaszi, 2011a).

Groups of five male and five female Wistar albino rats were orally dosed with glyphosate technical (purity 99%) at 2.5, 3.5, 5.0, 7.0 or 9.9 g/kg bw. The test material was administered as a 25% w/v solution in distilled water.

Of the 10 rats in each dose group, one died at 2.5 g/kg, one at 3.5 g/kg, three at 5.0 g/kg, eight at 7.0 g/kg and all 10 at 9.9 g/kg. Signs of toxicity included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy and faecal staining of the abdomen.

The acute oral LD_{50} in rats was 5.6 g/kg (95% confidence limits: 4.9–6.3 g/kg) (Heenehan, 1979a).

In an acute oral toxicity study five male and five female Sprague Dawley–derived albino rats were orally dosed with a formulation (described as a clear viscous amber liquid with a specific gravity of 1.214 g/mL) containing 62% isopropylamine glyphosate.

There were no deaths. There were no signs of toxicity in the males; four of the females had anogenital staining, and one of these four had diarrhoea and another, soft faeces. All the rats had fully recovered by day 3. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no gross abnormalities at necropsy.

The rat acute oral LD_{50} of a formulation containing 62% isopropylamine glyphosate was greater than 5000 mg/kg bw (Moore, 1999).

(b) Acute dermal toxicity

Rats

In an acute dermal toxicity study, five male and five female Sprague Dawley rats were dermally dosed with 2 000 mg/kg glyphosate technical (purity not reported), moistened with an unspecified amount of water before application, for 24 hours.

There were no deaths. Clinical signs during exposure consisted of piloerection and reduced activity. All the rats gained weight on days 0–7 after dosing and all, except a female that lost 30 g, gained weight on days 7–14 after dosing. No abnormalities were detected at necropsy.

The rat dermal LD_{50} of technical (purity not reported) glyphosate was greater than 2000 mg/kg bw (Cuthbert & Jackson, 1989b).

Five male and five female Sprague Dawley albino rats were dermally dosed with 5050 mg/kg glyphosate technical (two analyses: 96.40 and 96.71% purity), for 24 hours. The test material was moistened with deionized water at 0.284 mL/g test material and placed on the skin.

There were no deaths and no clinical signs. All the rats gained weight on days 0–7 after dosing; except for one female that lost 3 g of weight, all gained or maintained weight on days 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat dermal LD_{50} of technical (two analyses: 96.40 and 96.71%) glyphosate was greater than 5050 mg/kg bw (You, 2009b).

Five male and five female Sprague Dawley (Crj:CD) rats were dermally exposed to technical glyphosate (purity 95.68%) at a concentration of 2000 mg/kg. Appropriate amounts of finely ground test material were applied to a shaved 4 × 5 cm area of skin on each rat. Each site was then covered with a filter paper moistened with 0.5 mL deionized water. A control group of five male and five female rats was similarly treated without the test material.

Following a 24-hour exposure, there were no deaths and no clinical signs. All the rats gained weight on days 0–7 and 7–14 after dosing, and the weight gains were similar in the glyphosate-treated rats and the controls. There were no abnormalities at necropsy.

The rat dermal LD_{50} of technical (95.68%) glyphosate was greater than 2000 mg/kg bw (Komura, 1995c).

In an acute dermal toxicity study, five male and five female HanRcc:WIST(SPF) rats were dermally exposed to 2000 mg technical glyphosate (purity 96.66%) over a 24-hour exposure. The test material was formulated in purified water at a concentration of 0.5 g/mL and applied at a volume dose of 4 mL/kg.

There were no deaths or any clinical signs. There was no dermal irritation in males. Dermal irritation (slight erythema, scaling, scabs) was seen in four females from day 4, persisting to day 12 at the latest. All the males gained weight on days 1–8 and 8–15 after dosing. Two females had slight (0.6

and 1.7 g) weight losses on days 1–8, but all had good weight gains on days 8–15. No macroscopic findings were observed at necropsy.

The rat dermal LD_{50} of technical (purity 96.66%) glyphosate was greater than 2000 mg/kg bw (Simon, 2009b).

In a series of acute dermal toxicity studies using 2000 mg technical glyphosate (purity 97.52%, 95.23% or 96.6%), five male and five female CD/Crl:CD(SD) rats were dermally exposed over 24-hour periods (Haferkorn, 2009b). In each study, the test material was suspended (0.2 g/mL) in *aqua ad iniectabilia*. This suspension was applied to eight layers of gauze, which was placed on a 5 × 6 cm patch of intact skin site. The gauze was covered with a plastic sheet secured with adhesive plaster.

There were no deaths. There were no signs of toxicity. All the rats gained weight on days 0–8 and 8–15 after dosing. No skin irritation was observed. No pathological changes were observed at necropsy.

The rat dermal LD_{50} of technical glyphosate (purity 97.52%, 95.23% and 96.6%) was greater than 2000 mg/kg bw (Haferkorn, 2009b, 2010c,d).

Five male and five female Sprague Dawley–derived albino rats were dermally exposed to 5000 mg technical glyphosate (purity 97.23%) for 24 hours. The test material was mixed with distilled water to form a dry paste (70% w/w mixture in distilled water). An appropriate amount of this paste was applied to a 2 × 3 inch (about 5.1 × 7.6 cm) 4-ply gauze pad which was placed on the skin. The gauze pad and trunk of the rat were then wrapped with Durapore tape.

There were no deaths and no signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no abnormalities at necropsy.

The rat dermal LD_{50} of technical (97.23%) glyphosate was greater than 5000 mg/kg bw (Merkel, 2005b).

Five male and five female Wistar Hannover rats were dermally exposed to 2000 mg technical glyphosate (purity 98.05%) for 24 hours. The test material was placed on a porous gauze dressing moistened with deionized water. The gauze dressing was held on the skin with a non-irritating tape, and the test site and trunk of the animal covered with adhesive tape.

There were no deaths and no signs of toxicity. All the rats gained weight on days 0–7 after dosing and on days 7–14, with the exception of two females (one lost 2 g, the other maintained weight). There were no specific findings at necropsy.

The rat dermal LD_{50} of technical (980.5 g/kg) glyphosate was greater than 2000 mg/kg bw (Do Amaral Guimaraes, 2008b).

Five male and five female HanRcc:WIST(SPF) rats were dermally exposed to 2000 mg technical glyphosate (purity 95.1%) for 24 hours. The test material was diluted in PEG 300 to a concentration of 0.33 g/mL, and 6 mL/kg of this dilution was applied to intact, shaved skin and covered with a semi-occlusive dressing that was wrapped around the abdomen and fixed with an elastic adhesive bandage.

There were no deaths and no clinical signs were observed. All the rats gained weight on days 1–8 and 8–15 after dosing except for one female that maintained weight on days 8–15. There were no macroscopic findings at necropsy.

362

The rat dermal LD_{50} of technical (95.1%) glyphosate was greater than 2000 mg/kg bw (Talvioja, 2007b).

Five male and five female Alpk:APfSD (Wistar-derived) rats were dermally dosed with 2000 mg technical glyphosate acid (purity 95.6%) for 24 hours. The appropriate amount of test material was weighed out onto a plastic weighing boat and moistened to a dry paste with 0.6–0.8 mL deionized water before being applied onto approximately half of a 10 × 5 cm clipped area of skin. The amount of test material applied per unit area of exposed skin was about 20.0–21.9 mg/cm^2 for males and 16.2–17.3 mg/cm^2 for females. The paste was covered by a 4-ply gauze patch (about 7 × 7 cm) kept in contact with the skin for 24 hours using an occlusive dressing. The gauze patch was covered by a patch of plastic film held in place by an adhesive bandage (about 25 × 7 cm) secured by two pieces of PVC tape (about 2.5 × 20 cm).

None of the animals died and there were no significant signs of systemic toxicity. Some rats showed signs of urinary incontinence, but this is common in dermal toxicity studies because of bandaging and is not considered toxicologically significant. The skin of all rats was stained cream by the test material for up to 8 days, but there were practically no signs of skin irritation. One male had slight erythema on days 2–3 after dosing, and one female had small scabs on days 3–8 after dosing. All gained weight on days 1–8, and, with the exception of one female that lost 2 g, all gained weight on days 8–15 after dosing. At necropsy, the only finding was that one female had red mottled lungs, which was reported as common in rats of this age and strain and not considered treatment related.

The rat acute dermal LD_{50} of technical (95.6%) glyphosate acid was greater than 2000 mg/kg bw (Doyle, 1996b).

Five male and five female HanRcc:WIST(SPF) rats were dermally exposed to 5000 mg technical glyphosate acid (purity 96.1%) for 24 hours. The appropriate amount of test material was weighed out onto a plastic weighing boat and moistened to a dry paste with 0.5–0.6 mL purified water. The dry paste was applied evenly on an intact 8 cm^2 area of clipped skin which was covered with tape.

There were no deaths and no clinical signs were observed. All the rats gained weight on days 1–8 and 8–15. There were no macroscopic findings at necropsy.

The rat dermal LD_{50} of technical (95.6%) glyphosate acid was greater than 5000 mg/kg bw (Arcelin, 2007b).

Five male and five female Rj:Han (WI) Wistar rats were dermally exposed to 5000 mg technical glyphosate (purity 96.3%) for 24 hours. Sufficient water to moisten the test material was used to ensure good contact with the skin. The test material suspension was applied uniformly at the dermal site. Gauze pads were placed over the site, and these were covered with a hypoallergenic plaster. The entire trunk of the rat was then wrapped with semi-occlusive plastic wrap for 24 hours.

There were no deaths and no clinical signs were observed. There was no treatment-related dermal irritation. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no macroscopic observations at necropsy.

The rat dermal LD_{50} of technical (96.3%) glyphosate acid was greater than 5000 mg/kg bw (Zelenak, 2011a).

Rabbits

In an acute dermal toxicity study, five male and five female New Zealand White rabbits were dermally exposed to 5000 mg/kg bw glyphosate (purity 85.5%) for 24 hours. The test material was

applied dry to a strip of 8-ply gauze and then moistened with about 15 mL 0.9% saline. The gauze strip was then placed on the skin.

All the rabbits survived the 14-day observation period, with little or no change in body weights. No clinical signs were observed. There was no dermal irritation. Nothing remarkable was observed at gross necropsy.

The rabbit dermal LD_{50} of glyphosate (85.5%) was greater than 5000 mg/kg bw (Blaszcak, 1988b).

Five male and five female New Zealand White rabbits were dermally exposed to 5000 mg/kg glyphosate (purity 97.76%) for a 24-hour occluded exposure. The test material was moistened with 0.9% saline (about 1 mL/g of test material). An appropriate amount of this mixture was then applied to each application site.

One female rabbit died at 14 days, but this death was attributed to mucoid enteropathy and not to exposure to the test material. Other signs were anorexia, diarrhoea and soft stools. Most rabbits gained slight amounts of weight in the 14-day observation period. At necropsy, one male rabbit had a white caseous substance adhering to the lungs but this was not ascribed to exposure to the test material; otherwise, there was nothing remarkable.

The rabbit dermal LD_{50} of glyphosate (97.76%) was greater than 5000 mg/kg (Reagan, 1988a).

In an acute dermal toxicity study, two male and two female New Zealand White rabbits were dermally exposed (on abraded skin) to 5000 mg glyphosate technical (99%)/kg for a 24-hour occluded exposure. The test material was applied as a 25% w/v solution in physiological saline.

All the rabbits survived. All had a clear nasal discharge, which had cleared by day 6. One male lost weight over the 14-day observation period. At 24 hours, there was well-defined erythema in two rabbits and very slight erythema in the two others; two had very slight oedema. At necropsy, there were no internal or external abnormalities.

The rabbit dermal LD_{50} of glyphosate technical was greater than 5000 mg/kg (Heenehan, 1979b).

(c) Exposure by inhalation

In an acute inhalation toxicity study, five male and five female CD/Crl:CD(SD) rats were exposed (nose only) for 4 hours to a mean concentration (HPLC-determined) of 5.18 mg/L (5.05 mg/L as measured gravimetrically) with glyphosate technical (purity 96.6%).

There were no mortalities. All the rats exhibited tremors and dyspnoea, which remained for 3 hours after exposure (last observation on day 1); these effects were no longer present on test day 2 (the day following exposure). All the rats gained weight on days 0–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation median lethal concentration (LC_{50}) of glyphosate (purity 96.6%) was greater than 5.18 mg/L (Haferkorn, 2010e).

In an acute inhalation toxicity study, five male and five female F344/DuCrj(SPF) rats were exposed (whole body) for 4 hours to a mean concentration (determined analytically) of 5.48 mg/L glyphosate technical (purity 97.56%).

There were no deaths. All the rats' fur in the perioral and periocular regions was wet and stained red with sticky material, which disappeared by day 4 in males and by day 5 in females. All the rats gained weight on days 0–7 and 7–14 after dosing. No abnormalities were detected at necropsy.

The rat inhalation LC_{50} of technical (97.56%) glyphosate was greater than 5.48 mg/L (Koichi, 1995).

In an acute inhalation toxicity study, five male and five female HsdRccHan rats were exposed (nose only) to a mean concentration (gravimetrically determined) of 5.04 mg/L glyphosate technical (purity 96.66%).

There were no deaths. All the rats showed an increased respiratory rate, hunched posture, piloerection and wet fur; these signs were still present 1 hour after exposure but were gone the following day. All the rats gained weight on days 0–7 after dosing, and all gained or maintained weight on days 7–14 after dosing. There were no macroscopic observations at necropsy.

The rat inhalation LC_{50} of technical (96.66%) glyphosate was greater than 5.04 mg/L (Griffiths, 2009).

In an acute inhalation toxicity study of glyphosate technical (purity 97.52%), five male and five female CD/Crl:CD(SD) rats were exposed (nose only) to 5.12 mg/L (determined by HPLC).

There were no deaths. All the rats had slight dyspnoea and ataxia which were still present at 1 hour but not at 3 hours. All the rats gained weight on days 0–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (97.52%) glyphosate was greater than 5.12 mg/L (Haferkorn, 2009c).

In an acute inhalation toxicity study, five male and five female CD/Crl:CD(SD) rats were exposed (nose only) for 4 hours to a mean concentration (HPLC-determined) of 5.02 mg/L (4.99 mg/L measured gravimetrically) glyphosate technical (purity 95.23%).

There were no deaths. All rats showed slight ataxia, slight tremors and slight dyspnoea which were still present in all the animals at 3 hours (last observation on day 1) after exposure; these signs were no longer present on test day 2 (the day following exposure). All the rats gained weight on days 0–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (95.23%) glyphosate was greater than 5.02 mg/L (Haferkorn, 2010f).

In an acute inhalation toxicity study, five male and five female Sprague Dawley rats were exposed (nose only) for 4 hours to a mean concentration of 2.24 mg/L (nominal concentration: 7.89 mg/L) glyphosate (two batches: purity 96.40% and 96.71%).

There were no deaths. All the rats showed piloerection and activity decrease from 4.5 hours after exposure began until day 4. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate (two analyses: 96.40% and 96.71%) was greater than 2.24 mg/L (Carter, 2009).

In an acute inhalation toxicity study, five male and five female Sprague Dawley rats were exposed (nose only) for 4 hours to a gravimetrically determined mean concentration of 2.04 mg/L (nominal concentration: 8.99 mg/L) glyphosate technical acid (purity 97.23%).

365

There were no deaths or signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate acid technical (97.23%) was greater than 2.04 mg/L (Merkel, 2005c).

In an acute inhalation toxicity study, five male and five female rats (strain not reported: "healthy young adults supplied by BIOAGRI'S rearing house") were exposed (nose only) for 4 hours to a gravimetrically determined mean concentration of 5.211 mg/L glyphosate acid technical (purity 98.05%).

There were no deaths or signs of toxicity. All the rats gained weight on days 0–7 and 7–14 after dosing. There were no observable abnormalities at necropsy.

The rat inhalation LC_{50} of glyphosate acid technical (purity 98.05%) was greater than 5.211 mg/L (Dallago, 2008).

In an acute inhalation toxicity study, five male and five female HanRcc:WIST(SPF) rats were exposed (nose only) for 4 hours to a gravimetrically determined concentration of 3.252 mg/L (nominal: 6.304 mg/L) technical (purity 95.1%) glyphosate.

There were no deaths. Two males had salivation and rales following exposure, and another male had rales only. Two females had rales. All signs were gone two days after exposure. All gained weight on days 1–8 and 8–15 after dosing. There were no pathological findings at necropsy.

The rat inhalation LC_{50} of technical (95.1%) glyphosate was greater than 3.252 mg/L (Decker, 2007).

In an acute inhalation toxicity study, five male and five female Alpk:AP_fSD (Wistar derived) rats were exposed (nose only) for 4 hours to a particulate concentration of 4.43 mg/L glyphosate acid (purity 95.6%); the chemical concentration was 4.27 mg/L. Two males and one female exposed to 4.43 mg/L were found dead and one female was terminated in extremis; these events took place on days 5, 6 or 9 after dosing. Clinical signs seen in all rats included decreased activity, irregular breathing, hunched posture and piloerection. Signs observed in some rats included splayed gait, reduced stability, signs of urinary incontinence, gasping and vocalization. Hunched posture persisted in some females until day 13 after dosing. All the surviving males and females lost weight on days 1–8, but gained weight days on 8–15 after dosing.

The two males found dead had dark lungs, probably as a result of agonal congestion; the lungs of the decedent females were normal and the report states that the dark lungs in the males were probably the result of agonal congestion.

Because of the high mortality at 4.43 mg/L, a second group of five male and five female rats was exposed to a particulate concentration of 2.47 mg/L glyphosate acid (the chemical concentration was measured to be 2.43 mg/L). No mortality occurred in this group. Clinical signs seen in all rats included hunched posture, piloerection and salivation. All the males and four of the females had abnormal respiratory noise, which was still present in one male on day 15 after dosing. All the rats gained weight on days 1–8 and 8–15 after dosing. At necropsy one female had dark lungs and another had a few red spots on the lung. These were probably incidental observations,

The rat inhalation LC_{50} of glyphosate acid (95.6%) was greater than 4.43 mg/L, although mortality (in 4/10 rats) occurred at this concentration. No mortality occurred at 2.47 mg/L, although there were signs of toxicity (Rattray, 1996).

In an acute inhalation toxicity study, five male and five female Wistar RjHan (WI) rats were exposed (nose only) for 4 hours to a gravimetrically determined concentration of 5.04 mg/L (nominal: 7.71 mg/L) glyphosate technical (purity 96.9%). The percentage of aerosol that was less than 4 µm (considered the inhalable portion) was 54.4%.

One male was found dead on day 4. All the rats had laboured and noisy respiration, respiratory rate increase, gasping, sneezing, decreased activity and looked thin. All the surviving rats recovered by day 3; the male that died had slight noisy respiration, slight laboured respiration and a wasted appearance on day 3 (this animal had lost 47 g from day 0–3 after dosing). Specific cause of death was not determined. All the survivors gained weight on days 0–7 after dosing except for one male which lost 9 g; all gained weight on days 7–14. At necropsy, the male decedent had dark/red discolouration of the lungs and thymus. No observations were noted for the surviving rats.

The rat inhalation LC_{50} of glyphosate technical (96.9%) was greater than 5.04 mg/L, with one rat dying following exposure to this concentration (Nagy, 2011).

In an acute inhalation toxicity study of NUP5a99 (described as a clear viscous liquid containing 62% isopropylamine glyphosate and 31% other ingredients), five male and five female Sprague Dawley–derived albino rats were exposed (whole body) for 4 hours to a gravimetrically determined concentration of 2.08 mg/L (nominal value: 18.38 mg/L).

There were no deaths. In-chamber clinical observations included ocular and nasal discharge, hunched posture and hypoactivity, but the rats recovered quickly on removal from the chamber and the only finding 1 hour post-exposure was test material on the fur. All the rats gained weight on days 0–7 and 7–14 post dosing. There were no gross abnormalities at necropsy.

The inhalation LC_{50} of NUP5a99 glyphosate MUP (62% isopropylamine glyphosate) was greater than 2.08 mg/L (Wnorowski, 1999).

In an acute inhalation toxicity study of MON 78623 (47.2% glyphosate acid equivalent; 57.8% potassium salt of glyphosate), two groups of five male and five female Hsd:Sprague Dawley rats were exposed for 4 hours to either 2.21 or 5.27 mg/L glyphosate equivalent.

There were no deaths at either 2.21 or 5.27 mg/L. At 2.21 mg/L, breathing was congested and there was dark material around the eyes and/or nose, both of which cleared by day 8 after dosing. At 5.27 mg/L, the rats exhibited congested breathing, with reduced faecal output in two females on day 1. All signs of toxicity had cleared by day 3 after dosing. At 2.21 mg/L, all the rats gained weight on days 0–7 and 7–14 after dosing. At 5.27 mg/L all the males gained weight on days 0–7 and 7–14 after dosing, while two females (the ones with reduced faecal output on day 1) lost 2 and 6 g on days 0–7; another female lost 6 g on days 7–14; otherwise females gained weight on days 0–7 and 7–14 after dosing. At both 2.21 and 5.27 mg/L, none of the tissues showed any abnormalities at necropsy.

The inhalation LC_{50} of MON 78623 (47.2% glyphosate acid equivalent; 57.8% potassium salt of glyphosate) was greater than 5.27 mg/L (Bonnette, 2004).

(d) Dermal irritation

The results of studies of primary dermal irritation with glyphosate are summarized in Table 9.

In a dermal irritation study, three male and three female New Zealand White rabbits were dermally exposed for 4 hours to 0.5 g glyphosate technical (NUP 05068; purity 95.1%) mixed in about 0.5 mL purified water and applied to a 4 × 4 cm gauze patch that was placed on the skin. The patch was covered with a semi-occlusive dressing that was wrapped around the abdomen and anchored with tape.

All irritation scores were zero. The primary dermal irritation index (PDII) was zero. A 4-hour semi-occluded exposure to glyphosate technical (95.1%) over a skin area of about 16 cm^2 (rather than the usual 6 cm^2) resulted in no dermal irritation (Talvioja, 2007c).

In three separate dermal irritation studies, three male Himalayan rabbits per study were dermally exposed for 4 hours with 1000 or 2000 mg of glyphosate technical (purity 95.23%) (Leuschner, 2009a), glyphosate technical (purity 97.52%) (Leuschner, 2009c), or glyphosate technical (purity 96.6%) (Leuschner, 2010a) mixed with 0.5 (for 1000 g) or 1.0 mL (for 2000 g) *aqua ad iniectabilia*. This paste (750 mg, containing 500 mg glyphosate) was applied to a 6 cm^2 area of skin on each of the rabbits. The paste was covered with a gauze patch held in place with non-irritating hypoallergenic) tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour dermal exposure to glyphosate technical (purity 95.23%, 97.52% or 96.6%) resulted in no dermal irritation (Leuschner, 2009a,c, 2010a).

In a dermal irritation study, six female New Zealand White rabbits were dermally exposed for 4 hours to glyphosate technical (HR-001; purity 97.56%). The test material was finely ground in a mortar and 0.5 g put on a 2.5 × 2.5 cm area on each rabbit. A 2.5 × 2.5 cm gauze patch moistened with 0.5 mL water was then placed over the test material and held in place with a polyethylene sheet and non-irritating occlusive tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour exposure to HR-001 (97.56% active glyphosate) resulted in no dermal irritation (Hideo, 1995a).

In a dermal irritation study, one male and two female New Zealand White rabbits were dermally exposed for 4 hours to 500 mg glyphosate technical (purity 96.71%) moistened with 0.2 mL deionized water. This mixture was applied to each test site and covered with a 2.5 × 2.5 cm gauze patch. Each patch was secured in place with a strip of non-irritating adhesive tape. The entire trunk of each rabbit was loosely wrapped with a semi-permeable orthopaedic stockinette secured at both edges with strips of tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour exposure to glyphosate technical grade (96.71%) resulted in no dermal irritation (You, 2009c).

In a dermal irritation study, three male New Zealand White rabbits were dermally exposed for 4 hours to a 70% w/w mixture of glyphosate acid technical (97.23% active) in distilled water. Some of this paste (0.71 g) was placed on 1 × 1 inch (2.54 × 2.54 cm) 4-ply gauze pads which were applied to a 6 cm^2 area of intact skin on each rabbit. The pad and entire trunk of each rabbit were then wrapped with semi-occlusive 3-inch Micropore tape.

At 1 hour after exposure, one site scored 1 for erythema using the Draize scoring method; all other scores were zero. All scores were zero at 24, 48 and 72 hrs. The PDII was 0.08. A 4-hour exposure to glyphosate acid technical (97.23%) resulted in very slight dermal irritation (Merkel, 2005d).

In a dermal irritation study, three female New Zealand White rabbits were dermally exposed for 4 hours to glyphosate technical (purity 98.0%). A moistened gauze pad with 0.5 g test material was placed on a 6 cm^2 area of skin and held in place with an adhesive non-irritating tape.

All irritation scores at 1, 24, 48 and 72 hours after exposure were zero. The PDII was zero. A 4-hour dermal exposure to glyphosate technical (purity 98.05%) resulted in no dermal irritation (Canabrava Frossard de Faria, 2008a).

In a dermal irritation study, three male and three female New Zealand White rabbits were dermally exposed for 4 hours to 0.5 g glyphosate (purity 97.76%) moistened with 0.5 mL physiological saline and applied to two intact test sites per rabbit. The test sites were semi-occluded with a 1 × 1 inch (2.54 × 2.54 cm) gauze patch held in place with Micropore tape.

All irritation scores at 0.5, 24, 48 and 72 hours after exposure were zero. The PDII was 0.00. A 4-hour dermal exposure to glyphosate (purity 97.76%) resulted in no dermal irritation (Reagan & Laveglia, 1988b).

In a dermal irritation study, three male and three female New Zealand White rabbits were dermally treated for 24 hours with 0.5 mL glyphosate technical (purity 99%) as a 25% w/v solution in distilled water applied to four sites (two intact, two abraded) on each of six albino rabbits.

At 24 hours, one rabbit scored 1 for erythema at an intact site using the Draize scoring method and 1 for erythema and 1 for oedema at an abraded site. Another rabbit scored 1 for erythema at an abraded site. All other scores at 24 hours were zero. All scores for irritation at 72 hours after dosing were zero (Heenehan, 1979c).

In a dermal irritation study, 500 mg of glyphosate acid (purity 95.6%) was moistened with 0.5 mL of distilled water to form a dry paste that was applied to a 2.5 × 2.5 cm test site on the left flank of each of six female New Zealand White rabbits. The treated area was covered with an 8-ply 2.5 × 2.5 cm surgical gauze pad that was secured by two strips of surgical tape. This was covered by impermeable rubber sheeting that was wrapped once around the trunk of the animal and secured with adhesive polyethylene tape. Exposure was for 4 hours.

No irritation was observed at 30 minutes to 1 hour or 1, 2 or 3 days after dosing. All irritation scores were zero. The PDII was 0.00 (Doyle, 1996c).

In a dermal irritation study, 0.5 g of glyphosate technical (96.1% glyphosate acid) was moistened with about 0.5 mL purified water and placed on a 2.5 × 2.5 cm 8-ply gauze surgical patch that was applied to intact skin on the left flank of each of three male and three female New Zealand White rabbits. Each patch was covered with a semi-permeable dressing that was wrapped around the abdomen and held in place with tape. Exposure was for 4 hours.

No irritation was observed a 1, 24, 48 or 72 hours after dosing. All irritation scores were zero. The PDII was 0.00 (Arcelin, 2007c).

In a dermal irritation study, 0.5 g glyphosate technical (purity 96.3%) was dampened with water, and placed on a 2.5 × 2.5 cm surgical gauze pad that was kept on the skin of three male New Zealand White rabbits with hypoallergenic plaster for 4 hours. The entire trunk was wrapped with plastic wrap held in place with an elastic stocking.

One rabbit had grade 1 erythema at 1 and 24 hours after dosing. All other irritation scores were zero. The PDII was 0.17 (Zelenak, 2011b).

In a dermal irritation study, 0.5 g glyphosate wet cake (purity 85.5%) was moistened with 0.5 mL 0.9% saline and applied to the skin of six rabbits (two applications per rabbit). The applications were covered with 2.5 × 2.5 cm gauze squares for 4 hours of occluded exposure.

Five of the six rabbits showed grade 1 erythema at one or both sites at 0.5, 24 and/or 48 hours after dosing. All scores were zero at 72 hours. The PDII was 0.31 (Blaszcak, 1988c).

(e) Ocular irritation

The results of studies of primary eye irritation with glyphosate are summarized in Table 9.

In an eye irritation study, 0.1 g glyphosate technical (purity 95.1%) was instilled into the conjunctival sac of the left eye of each of three male and three female New Zealand White rabbits.

There was no iridial irritation (all irritation scores were zero). Corneal opacity along with positive conjunctival irritation (grade 2–3 redness and/or grade 2–3 chemosis) was in all the treated eyes at 1, 24 and 48 hours after dosing and in 2/3 treated eyes (with grade 2 redness) at 72 hours after dosing. On day 7 all scores for corneal opacity were zero; three eyes scored 1 (not considered a positive irritation effect) for conjunctival redness. All scores were zero on days 10 and 14.

Glyphosate technical (purity 95.1%) was considered to have caused significant but reversible damage to the rabbit eye (Talvioja, 2007d).

In eye irritation studies, 100 mg glyphosate technical (purity 95.23%, 97.52% or 96.6%) were instilled into the conjunctival sac of the right eye of each of three male Himalayan rabbits for each strength. An hour after instillation, the eyes were rinsed with 20 mL sodium chloride solution.

At purity 95.23%, corneal opacity (maximum score 1) was in all three eyes at 24, 48 and 72 hours after dosing; in two of the three eyes on day 4 after dosing; and in one of the three eyes on days 5, 6 and 7 after dosing; by day 8, clearing was complete. The maximum score for iritis was 1, which was observed in all three eyes at 24 hours, in two eyes at 48 hours, in one eye at 72 hours and in none of the eyes on day 4 and subsequently. The maximum score for conjunctival redness was 1, as was the maximum score for chemosis. All scores for conjunctival effects were zero by day 5. A fluorescein test at 24 hours showed corneal staining of between half and three quarters of the surface of two eyes, and in one quarter to half of the surface of one eye. A fluorescein test on day 7 showed corneal staining in one eye (up to one quarter of the surface).

At purity 97.52%, fluorescein testing at 24 hours showed corneal staining in two of the three eyes. At 24 and 48 hours, two eyes had corneal opacity and one of these still had corneal opacity at 72 hours. All the eyes had completely cleared (all eye irritation scores were zero) by day 4.

At purity 96.6%, all three eyes had corneal opacity at 24, 48 and 72 hours. At 4 days, two eyes had corneal opacity and one of these also had corneal opacity on day 5. All eyes had completely cleared (all eye irritation scores were zero) by day 7.

The three reports each concluded that "glyphosate TC was non-irritating to eyes, hence, no labelling is required" (Leuschner, 2009b, 2009d, 2010b).

In an eye irritation study of HR-001 (purity 97.56%), 0.1 g of the test material was placed in the conjunctival sac of the left eye of each of 12 female New Zealand White rabbits. Six rabbits (group A) did not receive an eyewash; three rabbits (group B) had their eyes washed out 30 seconds after instillation; and three rabbits (group C) had their eyes washed out 2 minutes after instillation.

All six rabbits in group A had corneal opacity through day 4. On day 7, five had corneal opacity. On day 21, three still had corneal opacity while the remaining three had completely cleared. In group B, all three rabbits had corneal opacity at 24 and 48 hours, but their eyes had completely

cleared (all scores were zero) by day 7. In group C, one rabbit was positive for corneal opacity at 24 hours, and none of the rabbits had corneal opacity at 48 hours. One group C rabbit was positive for conjunctival effects at 72 hours; the other two rabbits had completely cleared (all eye irritation scores were zero). None of the group C rabbit eyes was positive for irritation on day 4.

The report concluded that the test material had severely irritating potential for the eye mucosa of rabbits and that irrigation at 30 seconds or 2 minutes after application was effective for reduction of eye irritation and for recovery (Hideo, 1995b).

In an eye irritation study of glyphosate technical grade (two analyses: 96.40 and 96.71%), 0.1 mL (93.2 mg) was placed into the conjunctival sac of the right eye of each of two male and one female New Zealand White rabbits.

Of the three eyes, two still had corneal opacity at 24, 48 and 72 hours and at day 4. One eye had corneal opacity on day 7. All eyes had cleared by day 10.

The test material was rated as "moderately irritating and assigned to [United States Environmental Protection Agency; USEPA] Toxicity Category II" (You, 2009d).

In an eye irritation study of glyphosate acid technical (purity 97.23%), the test material was ground to a powder with a mortar and pestle and 0.1 mL (0.06 g) was instilled into the conjunctival sac of the right eye of three male New Zealand White rabbits. The pH of a 1% solution was reported as 2.5.

All three eyes were positive for corneal opacity through day 7, and for iritis and conjunctivitis through day 4 (one eye was also positive for conjunctival redness on day 7). All eyes had cleared (all irritation scores were zero) by day 10. According to the report,

> The Maximum Mean Total Score of Glyphosate Technical is 40.3. Based on the classification system used the test substance is considered severely irritating to the eye. The classification was raised from moderately to severely [irritating] because all three animals had scores greater than 10 on day 7 of the study (Merkel, 2005e).

In an eye irritation study, 0.1 g of glyphosate technical (purity 980.5 g/kg) was instilled in an eye of each of male and female New Zealand White rabbits. Because of the severity of the effects only two eyes were tested. The pH of a 1% solution is reported as 2.2.

In one rabbit there was corneal opacity, iritis and conjunctival effects through day 4 with clearing by day 7. In the other rabbit there was corneal opacity at 1, 24, 48 and 72 hours and at 7, 14 and 21 days after dosing. The eye was also positive for conjunctival irritation on day 14 after dosing (Canabrava Frossard de Faria, 2008b).

In an eye irritation study, 0.1 g glyphosate (purity 97.76%) was instilled in the conjunctival sac of one eye of each of six New Zealand White rabbits (sex not reported). The eyes were not washed out until 24 hours after instillation of the test material.

Corneal opacity and conjunctival irritation with blistering was observed in all the rabbits. One rabbit (which still had corneal opacity on day 14) was found dead at 20 days after instillation; the death was considered unrelated to exposure to the test material. Of the five surviving rabbits, three still had corneal opacity on day 21.

Because the glyphosate (97.76%) was severely irritating to the eye, it was assigned to USEPA Toxicity Category I for this exposure route (Reagan & Laveglia, 1988c).

371

In an eye irritation study of glyphosate acid (purity 95.6%), 100 mg was applied into the conjunctival sac of one female New Zealand White rabbit. This application caused moderate pain in this first rabbit so the other five animals were pre-treated with a local anaesthetic. Nevertheless, "between one quarter and one half of the test material was displaced from the eye of each animal immediately after dosing", according to the report (Johnson, 1997).

Corneal, iridial and conjunctival effects were seen in all rabbits for up to 4 days post dosing. Corneal opacity was seen in five of the six rabbits on day 4, but had cleared in all of them by day 7. All scores were 0 on day 7 except for one rabbit which had grade 1 (not considered positive for irritation) conjunctival redness, which had cleared by day 8.

Glyphosate acid (purity 95.6%) was classified as a mild irritant (class 5 on a 1–8 Draize scoring method) to the rabbit eye (Johnson, 1997).

In an eye irritation study, 0.1 g of glyphosate technical (purity 96.1%) was instilled into the conjunctival sac of the left eye of each of three New Zealand White rabbits. The pH of the test material was reported as 2.12.

There was no corneal opacity or iritis. All three rabbit eyes were positive for conjunctival irritation at 1 hour, and two were positive for these effects at 24, 48 and 72 hours after dosing. All scores were 0 by day 7.

The report concluded that "...the test item did not induce significant or irreversible damage to the rabbit eye" (Arcelin, 2007d).

In an eye irritation study, 0.1 g of glyphosate technical (purity 96.3%) was instilled into the conjunctival sac of the left eye of one male New Zealand White rabbit. The pH of the test material was reported as 1.99.

An Initial Pain Reaction score of 3 (on a scale of 0–5) was observed. Irritation effects were scored at 1 and 24 hours after instillation. According to the report (Tavaszi, 2011b):

> Conjunctival redness, chemosis and conjunctival discharge, as well as corneal opacity, were observed in the rabbit at 1 and 24 hours after application. Additionally, corneal erosion, redness of the conjunctiva with pale areas, pink, clean ocular discharge, oedema of the eyelids, and a few black points on the conjunctiva and dry surface of the eye were noted at one hour after the treatment. Fluorescein staining was positive at the 24 hour observation. Based on the symptoms, no further animals were dosed and the study was terminated after the 24 hour observation...
>
> Glyphosate Technical was classified as corrosive to the eye. (Tavaszi, 2011b).

In an eye irritation study of glyphosate wet cake (purity 85.5%), 0.1 mL (68.9 mg) was instilled into the lower conjunctival sac of the right eye of six New Zealand White rabbits. The eyes were not washed out until 24 hours after instillation.

All the rabbits showed positive irritation effects (corneal opacity and/or grade 2 chemosis and/or redness and/or iritis) at 1–48 hours after dosing, and two rabbits showed positive irritation effects at 72 hours. None of the eyes was positive for irritation on day 7. The report concluded that "Glyphosate Wet Cake produced moderate to severe but reversible ocular irritation in all animals... Five had iritis and corneal opacities" (Blaszcak, 1988d).

In an eye irritation study of MON 77945 (described as an amber liquid, pH 4.59, containing 46.6% glyphosate acid), 0.1 mL was instilled into one eye of each of six rabbits.

372

There were no positive irritation effects (one eye scored 1 for conjunctival redness at 1 hour, all other scores were zero). The report concluded that "under conditions of this study, MON 77945 produced very mild, transient ocular irritation" (Blaszcak, 1998e).

In an eye irritation study of MON 78623 (described as an amber liquid with 57.8% potassium salt of glyphosate; 47.13% glyphosate acid equivalent), 0.1 mL was instilled into an eye of each of three rabbits. Two rabbits vocalized following instillation.

There was no corneal opacity. At 1 hour, all eyes scored 1 for iritis, two for conjunctival redness and two for conjunctival swelling. At 24 hours, one eye scored 1 for iritis. All scores were zero at 48 hours. The report concluded that, "based on [European Economic Community] labelling criteria, MON 78623 is classified as a non-irritant to the ocular tissue of the rabbit" (Bonnette, 2001).

In an eye irritation study of MON 0139 (described as an amber liquid, with no information on pH or the active ingredient) 0.1 mL was instilled into an eye of each of nine rabbits. Six eyes were unwashed; three were washed out with physiological saline about 20 seconds after instillation.

All irritation scores were zero. No signs of irritation were observed in any rabbit eye (Branch, 1981).

In an eye irritation study of MON 8722 (described as a white powder, 90.8% purity, which was ground with a mortar and pestle prior to dosing), 0.1 g was instilled into an eye of each of six rabbits.

There was no corneal opacity or iritis. At 1 hour, conjunctival irritation (grade 2 redness and/or chemosis) was seen in five of the six eyes. At 24 and 48 hours, some of the eyes scored 1 for conjunctival redness. At 72 hours, all scores were zero (Busch, 1987a).

In an eye irritation study of MON 8750 (described as a white powder, 70.7% purity, which was ground with a mortar and pestle prior to dosing), 0.1 g (0.1 mL) was instilled into an eye of each of six rabbits.

There was no corneal opacity or iritis. At 1 hour, conjunctival irritation (grade 2 redness and/or chemosis) was seen in five of the six eyes. At 24 hours, one eye scored 1 for conjunctival redness (not considered a positive irritation effect). At 48 hours all scores were zero (Busch, 1987b).

In an eye irritation study, 0.1 mL of a 25% w/v solution of glyphosate technical (purity 99%), in distilled water was instilled into the conjunctival sac of an eye of each of nine rabbits. Six eyes were unwashed, while the other three were washed out for 1 minute with lukewarm water starting 20 seconds after instillation.

One unwashed eye and two washed eyes showed corneal opacity, with clearing by day 4. All scores were zero by day 7. In this study, glyphosate (purity 99%) was moderately irritating to the eye (Heenehan, 1979d).

In an eye irritation study, 0.1 g glyphosate (purity 97.76%) was instilled into the conjunctival sac of one eye of each of six rabbits. Corneal opacity and conjunctival irritation were noted in all rabbits at 24, 48 and 72 hours and on day 7.

One rabbit was found dead at 20 days; however, the death was considered unrelated to exposure. On day 21, three of the remaining five rabbits still showed corneal opacity. In this study, glyphosate (97.76%) was severely irritating to the eye (Reagan, 1988b).

(f) Dermal sensitization

Results of studies of skin sensitization with glyphosate are shown in Table 10.

Table 10. Results of skin sensitization studies with glyphosate

Species	Strain	Sex	Route	Purity (%)	Results	Reference
Mouse	CBA/Ca	F	LLNA	96.1	Negative	Betts (2007)
Mouse	CBA/J Rj	F	LLNA	96.3	Negative	Török-Bathó (2011)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	95.1	Negative	Talvioja (2007e)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	97.52	Negative	Haferkorn (2009d)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	Two analyses: 95.23 & 96.4	Negative	Haferkorn (2010g)
Guinea pig	Hartley	F	Magnusson–Kligman Maximization	97.56	Negative	Hideo (1995c)
Guinea Pig	Hartley	M	Magnusson–Kligman Maximization	96.66	Negative	Simon (2009d)
Guinea pig	Dunkin Hartley	M	Magnusson–Kligman Maximization	Two analyses: 97.52 & 98.8	Negative	Haferkorn (2010h)
Guinea pig	Short-haired Hartley albino	M + F	Buehler	Two analyses: 96.4 & 95.71	Negative	You (2009e)
Guinea pig	Hartley albino	M + F	Buehler	97.23	Negative	Merkel (2005f)
Guinea pig	Hartley	M	Buehler	98.05	Negative	Lima Dallago (2008)
Guinea pig	Dunkin Hartley	F	Magnusson–Kligman Maximization	95.7	Negative	Richeux (2006)
Guinea pig	Albino Crl (HA) BR	F	Magnusson–Kligman Maximization	95.6	Negative	Doyle (1996d)
Mouse	CBA/Ca	F	LLNA	96.1	Negative	Betts (2007)
Mouse	CBA/J Rj	F	LLNA	96.3	Negative	Török-Bathó (2011)

F: female; LLNA: local lymph node assay; M: male

Mouse

In a local lymph node assay, about 25 µL of a 10, 25 or 45% w/v preparation of glyphosate technical (96.1% glyphosate acid) in dimethyl sulfoxide (DMSO) was applied to the dorsal surface of each ear of groups of four female CBA/Ca mice. A vehicle control group was similarly treated with DMSO alone. The procedure was repeated daily for 3 consecutive days.

Three days after the third application, all the animals were injected in the tail vein with about 250 µL of phosphate buffered saline containing 20 µCurie (µCi; 74×10^{10} Bq) [methyl-^{3}H]thymidine. The mice were terminated after about 5 hours. The drained auricular lymph nodes were removed from

379

each animal and, together with the nodes from the other animals in that group, placed in a container of phosphate buffered saline.

Single cell suspensions were prepared by straining the lymph nodes from a single group through a 200-mesh stainless steel gauze. The cell suspensions were washed three times by centrifugation with about 10 mL phosphate buffered saline. Approximately 3 mL of 5% w/v trichloroacetic acid was added and, after overnight precipitation at 4 °C, the samples were pelleted by centrifugation and the supernatant was discarded. The cells were resuspended in approximately 1 mL of trichloroacetic acid, and the suspensions transferred to scintillation vials; 10 mL of scintillant was added prior to ß-scintillation counting.

The following disintegrations per minute were obtained: 0% (DMSO alone): 3912; 10%: 2394 (Stimulus Index or SI of 0.61 relative to vehicle control); 25%: 3292 (SI: 0.84); 45%: 508 (SI: 1.04). The following disintegrations per minute were obtained from the positive control (α-hexylcinnamaldehyde in 4 parts acetone and 1 part olive oil): 0%; (vehicle alone): 5939; 5%: 10 111 (SI: 1.70); 10%: 13 747 (SI: 2.31); and 25%: 38 015 (SI: 6.40, positive response > 3).

The study concluded that glyphosate technical material is not a skin sensitizer under these test conditions (Betts, 2007).

A local lymph node assay of glyphosate technical (96.3%) used groups of four female CBA/J Rj mice with each mouse topically dosed on the dorsal surface of each ear with 25 µL of 10%, 25% or 50% w/v preparation of glyphosate technical in propylene glycol, propylene glycol alone or 25% α-hexylcinnamaldehyde in propylene glycol. The procedure was repeated daily for three consecutive days.

Three days after the third application, all the animals were injected in the tail vein with about 250 µL of phosphate buffered saline containing 20 µCi (74×10^{10} Bq) [methyl-^{3}H]thymidine. The mice were terminated about 5 hours later. The draining auricular lymph nodes were removed from each animal and, together with the nodes from the other animals in that group, placed in a Petri dish containing 1–2 mL phosphate buffered saline.

Single cell suspensions of pooled lymph node cells were prepared and collected in tubes by gentle mechanical disaggregating of the lymph nodes through a cell strainer. The cell strainer was washed with phosphate buffered saline. Pooled lymph node cells were pelleted in a centrifuge at about 190 g for 10 minutes at 4 °C. Afterwards, the centrifugation supernatants were discarded. The pellets were gently resuspended and 10 mL phosphate buffered saline added to the tubes. The washing step was repeated twice. This was repeated for each group of pooled lymph nodes. After the final washing, suspensions were centrifuged and most of the supernatant was removed except for a small volume (< 0.5 mL) above each pellet. Each pellet was resuspended in 3 mL of 5% trichloroacetic acid. After an 18-hour incubation with 5% trichloroacetic acid at 2–8 °C, the precipitate was recovered by centrifugation at 190 g for 10 minutes. The supernatants were removed and the pellets resuspended in 1 mL of 5% trichloroacetic acid solution and dispersed using an ultrasonic water-bath. Each precipitate was transferred to a scintillation vial with 10 mL of scintillation liquid and thoroughly mixed prior to ß-scintillation counting.

The following disintegrations per minute were obtained after accounting for the background: 0% (propylene glycol alone): 681; 10%: 794 (Stimulus Index or SI of 1.2 relative to vehicle control); 25%: 678 (SI: 1.0); 50%: 683 (SI: 1.0); positive control (α-hexylcinnamaldehyde in propylene glycol): 25%: 8302 (12.2 SI, positive response > 3).

The study concluded that under the conditions of this local lymph node assay, glyphosate technical had no skin sensitization potential (i.e. it was a non-sensitizer) (Török-Bathó, 2011).

Guinea pigs

In a Magnusson–Kligman maximization test with female Dunkin Hartley guinea pigs, intradermal induction treatments were with a 3% dilution of glyphosate technical (95.1%) in PEG 300 and in an emulsion of Freund's Complete Adjuvant/physiological saline. Epidermal induction (1 week after the intradermal induction) was for 48 hours under occlusion with the test material at 50% in PEG 300. Two weeks later, the five control and 10 test guinea pigs were challenged. Patches (3 × 3 cm) of filter paper were saturated with about 0.2 mL of the test material at the highest tested non-irritating concentration of 25% in PEG 300 (applied to the left flank) and about 0.2 mL PEG 300 alone (applied to the right flank) for 24 hours. The application sites were scored at 24 and 48 hours after exposure ended.

All challenge irritation scores (for the 10 test and five control animals) were zero. A positive control assay with α-hexylcinnamaldehyde gave appropriate results. Based on these findings, glyphosate technical does not have to be classified and labelled as a skin sensitizer (Talvioja, 2007e).

In a Magnusson–Kligman maximization test with female Dunkin Hartley guinea pigs, intradermal induction treatments were with a 0.01% concentration of glyphosate technical (two analyses: 95.23% and 96.4%) in *aqua ad iniectabilia*. The day before topical induction, the application site was treated with 0.5 mL sodium lauryl sulfate 10% in Vaseline. Topical induction (1 week after the intradermal induction) was 2 mL of a 50% concentration of glyphosate technical in *aqua ad iniectabilia* applied for 48 hours. The challenge, 2 weeks after the intradermal induction, was 2 mL of the test material placed on a filter paper on the left flank of each guinea pig; a filter paper with 2 mL vehicle was placed on the right flank as a control. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and five control guinea pigs) were zero. A positive control assay with benzocaine gave the expected results. Glyphosate technical (purity 95.23% and 96.4%) was determined to be not sensitizing to guinea pigs (Haferkorn, 2010f).

In a Magnusson–Kligman maximization test with female Hartley guinea pigs, a 5% suspension of glyphosate technical (purity 97.56%) in paraffin oil was intradermally injected. Six days later, the treatment site was treated with 10% sodium lauryl sulfate in white petrolatum; the topical induction (the following day) was with 0.4 g of the test material preparation (25% test material in white petrolatum) on a 2 × 4 cm piece of filter paper for 48 hours. The challenge application (2 weeks after the topical induction) was 25% test material in white petrolatum for 24 hours, with scoring at 24 and 48 hours following the end of this exposure.

None of the 20 induced guinea pigs and none of the 10 negative control guinea pigs showed any signs of irritation at the application site following challenge. A positive control assay with 2,4-dinitrochlorobenzene gave appropriate results.

The study concluded that glyphosate technical (purity 97.56%) had no dermal sensitization potential in guinea pigs (Hideo, 1995c).

In a Magnusson–Kligman maximization test with male Hartley guinea pigs, a 10% w/v dilution of glyphosate technical (purity 96.66%) in purified water and Freund's Complete Adjuvant was intradermally injected. Seven days later the application site was treated with the test material at 50% in purified water (about 0.3 mL applied on a 2 × 4 cm filter paper) with 48-hour exposure. Two weeks later, the guinea pigs were treated with the test material at 15% in purified water (about 0.2 mL applied on a 3 × 3 cm filter paper) for 24 hours, with scoring at 24 and 48 hours following the end of this exposure.

None of the 10 induced guinea pigs and none of the five control guinea pigs showed any signs of irritation at the application site following challenge. A positive control assay with α-hexylcinnamaldehyde gave the appropriate results.

Based on the results of this study, there is no sensitization potential of glyphosate technical (purity 96.66%) in the guinea pig (Simon, 2009d).

In a Magnusson–Kligman maximization test with male Dunkin Hartley guinea pigs, intradermal inductions were with a 0.5% concentration of glyphosate technical (purity 97.52% and 98.8%) in *aqua ad iniectabilia*. The day before the topical induction, the application site was treated with 0.5 mL sodium lauryl sulfate 10% in Vaseline. Topical induction (1 week after the intradermal induction) was 2 mL of a 50% concentration of glyphosate technical in *aqua ad iniectabilia* for 48 hours. The challenge was two weeks after the intradermal induction. Filter paper with 2 mL of the test material was placed on the left flank; as a control, a filter paper with 2 mL of the vehicle was placed the right flank. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and five control guinea pigs) were zero. A positive control assay with benzocaine gave the expected results. Glyphosate technical (purity 97.52% and 98.8%) was found to be not sensitizing to guinea pigs (Haferkorn, 2009d).

In a dermal sensitization (Magnusson–Kligman maximization test with male Dunkin Hartley guinea pigs), intradermal induction was with a 0.5% concentration of glyphosate technical (purity 96.6% and 97.3%) in *aqua ad iniectabilia*. The day before the topical induction, the application site was treated with 0.5 mL sodium lauryl sulfate 10% in Vaseline. The topical induction (1 week after the intradermal induction) was 2 mL of a 50% concentration of the test material in *aqua ad iniectabilia* for 48 hours. The challenge was two weeks later: filter paper with 2 mL of the test material was applied to the left flank for 48 hours, with filter paper with 2 mL of the vehicle applied to the right flank. The period of exposure was 24 hours, with scoring at 24 and 48 hours after removal of the filter papers.

All challenge irritation scores (for the 10 test and five control guinea pigs) were zero. A positive control assay with benzocaine gave the expected results. Glyphosate technical (purity 96.6% and 97.3%) was found to be not sensitizing to guinea pigs (Haferkorn, 2010g,h).

In a Buehler method dermal sensitization study with glyphosate technical (purity 96.40% and 96.71%), 15 male and 15 female short-haired Hartley albino guinea pigs were divided into two groups: group I (five males and five females) and group II (10 males and 10 females). For each induction treatment, 400 mg of the test material was placed on a four-ply 2.5 × 2.5 cm gauze pad and moistened with 2 mL deionized water. Each gauze pads was secured with non-irritating adhesive tape, which in turn was covered with a strip of clear polyethylene film. Exposures lasted for at least 6 hours and took place on days 1, 8 and 15. Group I animals were untreated during this period. After a 2-week rest period, all animals (groups I and II) were challenged at a previously unexposed site with 400 mg test material moistened with 2 mL deionized water.

All challenge irritation scores (for the 20 induced and 10 control guinea pigs) were zero. A positive control assay with α-hexylcinnamaldehyde gave the expected results. Glyphosate technical (purity 96.40% and 96.71%) did not elicit a sensitizing reaction in guinea pigs (You, 2009c).

In a Buehler method dermal sensitization study, a group of 20 male and 20 female Hartley albino guinea pigs were exposed once a week to 0.4 g of 70% w/w glyphosate acid technical (purity 97.23%) in distilled water. The mixture was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber, which was secured in place and wrapped with non-allergenic

adhesive tape. After each 6-hour exposure, the chambers were removed and any residual test material gently cleansed off. Twenty-seven days after the first induction dose, 0.4 g of a 70% w/w mixture of the test material in distilled water was applied to a naive site on the right side of each guinea pig. These sites were evaluated and scored approximately 24 and 48 hours after the challenge application. A group of 10 controls was similarly treated with the vehicle alone.

There were no positive irritation scores (defined as > 0.5). A positive control assay with α-hexylcinnamaldehyde gave the expected results. Glyphosate technical (97.23%) is not considered a contact sensitizer (Merkel, 2005f).

In a dermal sensitization study (Buehler method), a group of 20 male Hartley guinea pigs were treated three times with once-a-week 6-hour exposures to 1.0 mL of a 50% w/v solution of glyphosate technical (purity 98.05%) in a DMSO vehicle. The solution was applied in a cotton lint patch which covered approximately 6 cm^2 of the left flank. A group of 10 control guinea pigs was similarly treated with 1.0 mL DMSO. Two weeks after the last induction treatment, both induced and control guinea pigs were exposed for 4 hours to 1.0 mL of a 50% w/v solution of test material in DMSO on the right flank.

One of the 20 induced guinea pigs had a score of 1 (positive response) at 24 and 48 hours following challenge. All of the other induced and control animals scored zero.

The study concluded that the epidermal application of glyphosate technical (purity 98.05%) with DMSO as vehicle does not cause skin sensitization in guinea pigs according to the Buehler test method (Lima Dallago, 2008).

In a dermal sensitization (Magnusson–Kligman maximization test) study with glyphosate technical (purity 95.7%), the hair was clipped from an area approximately 4 × 6 cm on the shoulder region of each of a group of 20 female Dunkin Hartley guinea pigs on day 0. A row of three injections (0.1 mL each) was made on each side of the spine. The injections were: a) 1:1 Freund's Complete Adjuvant in isotonic sodium chloride; b) a 0.195% (v/v) formulation of the test material in isotonic sodium chloride; c) a 0.195% (v/v) formulation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus isotonic sodium chloride. On day 6, the scapular region was treated with 10% sodium lauryl sulfate (10% in petroleum jelly). On day 7 the same area used for the intradermal injections was treated with a 60% w/w mixture of the test material in distilled water, with 48-hour occluded exposure. The challenge was approximately 2 weeks later. One site was treated with 60% w/w mixture of the test material in distilled water; a second site was treated with a 30% w/w mixture of the test material in distilled water. The sites were scored for irritation at 24 and 48 hours following exposure.

A group of 10 control guinea pigs was similarly treated using the vehicle only during the induction period.

All 18 induced guinea pigs (2 had died during the study) scored zero at 24 and 48 hours following challenge, as did all 10 controls. The study reported that glyphosate technical (95.7) produced a 0% (0/18) sensitization rate and was classified as a non-sensitizer to guinea pig skin under the conditions of the test (Richeux, 2006).

In a dermal sensitization (Magnusson–Kligman maximization test) with glyphosate acid (purity 95.6%), a group of albino Crl (HA) BR guinea pigs each had the hair clipped from an area about 5 × 5 cm on the scapular region. A row of three injections (0.05–0.1 mL each) was made on each side of the spine. The injections were: a) 1:1 Freund's Complete Adjuvant in deionized water; b) a 0.1% (w/v) preparation of the test material in deionized water; c) a 0.% (w/v) preparation of the test material in a 1:1 preparation of Freund's Complete Adjuvant plus deionized water. On day 6 the application site was clipped and 0.5 mL of a 10% preparation of sodium lauryl sulfate in paraffin wax

applied. On day 7 the test area was treated with a topical application of the test material (75% w/v) in deionized water. The preparation (0.2–0.3 mL) was put on a 4 × 2 cm piece of filter paper held in place with surgical tape. The filter paper was covered by a strip of adhesive tape secured using self-adhesive PVC tape. This occlusive dressing was kept in place for about 2 days. Ten control animals were similarly treated with deionized water. Challenge (for both the induced animals and their controls) was at approximately 21 days. An area about 15 × 15 cm on both flanks of the test and control animals was clipped free of hair. An occlusive dressing was prepared using two pieces of approximately 1 × 1.75 cm filter paper stitched to a piece of rubber sheeting (about 12 × 5 cm). A 75% w/v preparation of the test material in deionized water (0.05–0.1 mL) was applied to a piece of filter paper and a 30% w/v preparation in deionized water (0.05–0.1 mL) applied to the second. These were covered with strips of adhesive bandage (about 25–40 cm × 7.5 cm) and secured with a self-adhesive PVC tape. Exposure was for about 24 hours. The sites were scored for irritation at 24 and 48 hours following the end of exposure.

Exposure to the 75% w/v preparation resulted in mild and scattered redness (score of 1) in three of the 20 induced and one of the 10 control animals at 24 hours only, with all scores zero at 48 hours. Because the redness was observed at similar incidences in both induced and control guinea pigs and because it occurred only at 24 hours, it was considered to be due to skin irritation rather than the test material. All sites exposed to the 30% w/v preparation scored zero at both 24 and 48 hours. A positive control assay with α-hexylcinnamaldehyde demonstrated the sensitivity of the test system.

The study concluded that glyphosate acid is not a skin sensitizer under the test conditions (Doyle, 1996d).

2.2 Short-term studies of toxicity

(a) Oral administration

Mice

In a 13-week oral toxicity study, groups of 15 male and 15 female CD-1 mice were fed diets containing glyphosate (purity 98.7%) at dietary concentrations of 0, 5000, 10 000 or 50 000 ppm (0, 944, 1870 and 9710 mg/kg bw per day for males and 0, 1530, 2740 and 14 800 mg/kg bw per day for the females, respectively).

There was no treatment-related mortality or clinical signs of toxicity, organ-weight change, macroscopic and histopathological findings. At study termination, body-weight gains of the males and females at 50 000 ppm were about 24% and 18% lower, respectively, than that of the control animals. Body-weight gains of both males and females at 5000 ppm and 10 000 ppm were comparable to those of the controls.

The no-observed-adverse-effect level (NOAEL) in the 13-week toxicity study in mice was 10 000 ppm (equal to 1870 mg/kg bw per day) based on reduced body weights at 50 000 ppm (equal to 9710 mg/kg bw per day) (Tierney & Rinehart, 1979).

In a 13-week oral toxicity study, groups of 10 male and 10 female CD-1 mice were fed diets containing glyphosate (purity 99.5%) at a concentration that was adjusted weekly to give doses of 200, 1000 or 4500 mg/kg bw per day. The animals were observed daily for symptoms of ill health and mortality. Body weights and feed consumption were recorded weekly, and water consumption was monitored throughout the study. Ophthalmoscopic examinations were performed during week 12. Blood samples were collected from the orbital sinus for haematology (seven parameters) and from the dorsal aorta at necropsy for clinical chemistry analysis (16 parameters). However, the small sample volumes precluded analysis of total protein, albumin and cholesterol. All the animals were terminated and necropsied, 13 organs were isolated and weighed, and about 35 separate tissues were fixed for microscopy. All tissues from animals in the highest dose group and in the control group and the

kidneys, liver and lungs of animals at the lowest (200 mg/kg) and intermediate (1000 mg/kg) doses underwent a full histopathological examination.

No treatment-related mortalities, clinical signs, haematological or biochemical findings and no organ-weight changes were observed. Gross or histopathological examination did not show any effects of glyphosate administration.

Taking into account the limited range of clinical chemistry parameters evaluated, the NOAEL in the 13-week toxicity study in mice was 4500 mg/kg bw per day, the highest dose tested in this study (Perry et al., 1991a).

In a 13-week oral toxicity study, groups of 10 male and 10 female B6C3F1 mice were fed diets containing glyphosate (purity 99%) at concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm (equal to 0, 507, 1065, 2273, 4776 and 10 780 mg/kg bw per day for males and 0, 753, 1411, 2707, 5846 and 11 977 mg/kg bw per day for females). All tissues from the highest-dose and control animals were examined microscopically. The salivary glands were also examined in all groups receiving lower doses.

Reduced body-weight gain was observed at 25 000 and 50 000 ppm in both males and females. There were no differences in feed consumption between control and treated mice. The only significant gross finding in the study was a "dark" salivary gland in a male at the highest dose; no other gross abnormalities were observed at necropsy. Histological changes were observed only in the parotid salivary gland (Table 11). The cytoplasmic alterations consisted of a diffuse increase in the basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared to be enlarged and had fewer ducts. No histological changes were observed in the submandibular and sublingual glands.

Table 11. Incidence and severity of cytoplasmic alteration of the parotid and submandibular salivary glands (combined) in mice administered glyphosate for 13 weeks

	No. of cases per dietary concentration of glyphosate					
	0 ppm	**3 125 ppm**	**6 250 ppm**	**12 500 ppm**	**25 000 ppm**	**50 000 ppm**
Males	0/10	0/10	5/10 (1.0)	9/10 (1.6)	10/10 (2.8)	10/10 (4.0)
Females	0/10	0/10	2/10 (1.0)	9/10 (1.3)	10/10 (2.4)	10/10 (3.1)

no.: number; ppm: parts per million

Results presented as number of mice showing cytoplasmic alterations / total number of mice in the group, with average severity score in parentheses. Severity score is based on a scale of 1 = minimal, 2 = mild, 3 = moderate or 4 = marked.

Source: Chan & Mahler (1992)

The NOAEL in the 13-week toxicity study in mice was 3125 ppm (equal to 507 mg/kg bw per day) based on parotid salivary gland lesions at 6250 ppm (equal to 1065 mg/kg bw per day) (Chan & Mahler, 1992).

In a 13-week oral toxicity study, groups of 12 male and 12 female ICR(Crj:CD-1)SPF mice were administered glyphosate (purity 97.56%) at dietary concentrations of 0, 5000, 10 000 or 50 000 ppm (equal to a mean daily glyphosate intake of 0, 600, 1221 and 6295 mg/kg bw per day for males and 0, 765, 1486 and 7435 mg/kg bw per day for females).

There were no treatment-related clinical signs, mortality or ophthalmological and haematological findings. At 50 000 ppm, mean body weights of the males were 91% that of the controls from week 2 to the end of the treatment; body weights of females were comparable to that of the controls. Similarly, feed consumption was slightly decreased in males at the highest dose. At

50 000 ppm, feed efficiency of males and females was lower than that of the controls at almost all measuring points during the treatment.

At 50 000 ppm, females showed a significant treatment-related increase in creatine phosphokinase ($P < 0.01$). Other statistically significant ($P < 0.01$) changes in clinical chemistry were observed in high-dose male and female mice; however, these changes were minor and not associated with any histological findings and not considered adverse. In all treated groups, males showed a significant decrease in urinary pH. There were no abnormalities in females of any treated groups.

At 50 000 ppm, males and females showed significant ($P < 0.01$) increases in both absolute and relative caecum weights (238% and 263%, respectively, for males, and 187% and 195%, respectively, for females) (Table 12).

Table 12. Caecum weights of mice administered glyphosate for mice 13 weeks

	Absolute and relative weight per dietary concentration of glyphosate			
	0 ppm	**5 000 ppm**	**10 000 ppm**	**50 000 ppm**
Males				
Absolute weight ± SD (mg) [a]	624 ± 86	609 ± 116	718 ± 177	1 484 ± 359
Relative weight ± SD (%)	1.45 ± 0.19	1.38 ± 0.26	1.61 ± 0.33	3.82 ± 1.15**
Females				
Absolute weight ± SD (mg) [a]	497 ± 96	474 ± 115	604 ± 123	958 ± 163**
Relative weight ± SD (%)	1.43 ± 0.26	1.37 ± 0.30	1.67 ± 0.42	2.79 ± 0.53**

ppm: parts per million; SD: standard deviation; **: $P < 0.01$

Relative weight expressed as (organ weight / body weight) × 100.

[a] At 50 000 ppm, both males and females showed significant increases in absolute weights (238% for males and 187% females).

Source: Kuwahara (1995)

At 50 000 ppm, males and females showed a significant increase in incidence of distension of the caecum (12/12 males and 10/12 females, in contrast to none in the control group). In addition, at this dose males showed significant increases in incidence of cystitis (4/12 compared to none in the control group). There were no significant changes in incidence in females. Although significant increases in incidence of distension of the caecum were noted for males and females at necropsy, histopathological examinations failed to reveal any abnormalities in the caecum.

The NOAEL in the 13-week toxicity study in mice was 10 000 ppm (equal to 1221 mg/kg bw per day) based on the decrease in body weights in males, increase in absolute and relative caecum weights in both sexes and increased incidence of distension of the caecum in both sexes at 50 000 ppm (equal to 6295 mg/kg bw per day) (Kuwahara, 1995).

Rats

In a 4-week range-finding study of oral toxicity, groups of five male and five female Sprague Dawley rats were fed diets containing glyphosate (purity 97.7%) at concentrations of 0, 30 000, 40 000 or 50 000 ppm (equivalent to approximately 1500, 2000 and 2500 mg/kg bw per day).

No animals died during the study. The only clinical signs of toxicity were soft stools and/or diarrhoea, which occurred in both sexes at all doses with diarrhoea being the predominant sign in animals at the highest dose during the last 3 weeks of the study. Slightly reduced body-weight gains were noted in both sexes at all the doses, although significant reductions consistently occurred only in males and females at the highest dose (9.6% and 9.0%, respectively, after 4 weeks). Daily feed

consumption was reduced for males at the intermediate and highest dose during the first week of the study. Feed intake for treated females was comparable to that of controls throughout the study. The only clinical signs of toxicity were soft stools and/or diarrhoea, which occurred in both sexes at all doses with diarrhoea being the predominant sign in animals at the highest dose during the last 3 weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions.

Because of the frequent occurrence of soft stools and/or diarrhoea at all doses, no NOAEL could be derived from this 4-week dietary toxicity study in rats (Reyna & Thake, 1989).

In a 4-week oral toxicity study, groups of five male and five female Sprague Dawley rats were fed diets containing glyphosate (purity 99.5%) at a concentration that was adjusted weekly to give doses of 0, 50, 250, 1000 or 2500 mg/kg bw per day. All the animals were terminated and necropsied, and the livers, hearts, kidneys, spleens and adrenals of control and highest-dose animals processed and examined histopathologically. Examination was subsequently extended to include the kidneys from all females in all the groups.

Soft faeces were noted in three males in the highest-dose group during weeks 3 to 4, but not in any other group. No treatment-related effects were observed on mortality, clinical signs of toxicity, body weights, feed and water consumption or haematological parameters. In males, equivocal increases in plasma alanine transaminase [alanine aminotransferase] and alkaline phosphatase activities were observed at 250, 1000 or 2500 mg/kg bw. In females, plasma alanine transaminase activity was significantly increased at the highest dose, as was total bilirubin. In addition, increased plasma concentrations of phosphate were noted in males at 1000 or 2500 mg/kg bw. There were neither notable intergroup differences in organ weights nor gross pathological findings. However, an increase in the incidence of very mild to slight nephrocalcinosis was observed in female rats at 250 mg/kg bw and higher doses (Table 13).

Table 13. Nephrocalcinosis in rats administered glyphosate for 4 weeks

	No. per dietary concentration of glyphosate									
	Males					Females				
	0 mg/kg bw per day	50 mg/kg bw per day	250 mg/kg bw per day	1 000 mg/kg bw per day	2 500 mg/kg bw per day	0 mg/kg bw per day	50 mg/kg bw per day	250 mg/kg bw per day	1 000 mg/kg bw per day	2 500 mg/kg bw per day
No. of cases	0	NI	NI	NI	NI	0	0	2	2	4
No. of very mild/minimal cases	0	NI	NI	NI	NI	0	0	1	1	2
No. of mild/slight cases	0	NI	NI	NI	NI	0	0	1	1	2

bw: body weight; NI: not investigated; no.: number

Source: Atkinson et al. (1989)

The NOAEL in the 4-week dietary toxicity study in rats was 50 mg/kg bw per day for slight nephrocalcinosis in female rats at 250 mg/kg bw per day (Atkinson et al., 1989). This finding was not confirmed in a separate study by Perry et al., 1991b.

In a 90-day oral toxicity study, groups of 12 male and 12 female Sprague Dawley rats were fed diets containing glyphosate (purity 95.2%) at concentrations of 0, 1000, 5000 or 20 000 ppm (calculated mean intakes equal to 0, 63, 317 and 1267 mg/kg bw per day for males and 0, 84, 404 and 1623 mg/kg bw per day for females). Clinical signs, body weight, feed consumption, haematology

and clinical chemistry parameters were monitored routinely. Gross examinations were performed for all groups, and the kidneys, liver and testes weighed after termination. A standard range of tissues from control and highest-dose animals was microscopically examined as well as the kidneys, livers and lungs from animals at all doses.

No treatment-related effects were observed at up to the highest dose. However, parotid salivary glands were not included in the histopathological examination.

The NOAEL in the 90-day dietary toxicity study in rats was 20 000 ppm (equal to 1267 mg/kg bw per day), the highest dose tested (Stout & Johnson, 1987).

In a 13-week oral toxicity study, groups of 10 male and 10 female Sprague Dawley rats were fed diets containing glyphosate (purity 98.6%) at concentrations that were adjusted weekly to doses of 0, 30, 300 or 1000 mg/kg bw per day. All tissues from control and highest-dose animals, in addition to the kidneys, liver, lungs and parotid salivary glands of all the test animals, underwent a full histopathological examination.

There were no mortalities, clinical signs or changes in body or organ weights, feed and water consumption, haematological parameters and ophthalmoscopic and macroscopic findings. Females at the highest dose showed slight but statistically significant increases in concentrations of glucose (11%; $P < 0.05$), total protein (9%; $P < 0.001$), albumin (9%; $P < 0.05$) and creatinine (8%; $P < 0.01$) compared with those in the control group. Urinalysis revealed a reduction in pH in males at the highest dose.

In contrast to results from a 4-week study in rats conducted at the same testing facility (Atkinson et al., 1989), the incidence of nephrocalcinosis in this 13-week study was evenly distributed in dose groups and sexes and was not dose dependant; it is therefore clearly not treatment related.

An increase in the incidence of cellular alterations (deep basophilic staining and enlargement of cytoplasm) was observed in the parotid salivary glands of both sexes in all treated groups. In addition, the severity (graded as very mild, mild, moderate, severe and very severe) of these findings showed a dose-related increase, but only reached statistical significance in males at the highest dose (Table 14), suggesting these changes are of equivocal toxicological significance.

Table 14. Cytoplasmic alteration of the parotid salivary gland in rats administered glyphosate in the diet for 13 weeks

	No. per dietary concentration of glyphosate							
	Males				Females			
	0 mg/kg bw per day	30 mg/kg bw per day	300 mg/kg bw per day	1 000 mg/kg bw per day	0 mg/kg bw per day	30 mg/kg bw per day	300 mg/kg bw per day	1 000 mg/kg bw per day
Severity [a]								
Very mild	3	7	6	0	2	7	7	1
Mild	0	0	3	2	0	1	2	4
Moderate	0	0	1	3	0	0	0	3
Severe	0	0	0	5*	0	0	0	1
Total incidence	3	7	10**	10**	2	8*	9**	9**

bw: body weight; no.: number; *: $P < 0.05$; **: $P < 0.01$

[a] Severity graded as very mild, mild, moderate, severe and very severe.

Source: Perry et al. (1991b)

The NOAEL in this 90-day toxicity study in rats was 300 mg/kg bw per day based on the more pronounced severity of cellular alterations in the parotid salivary gland at 1000 mg/kg bw per day (Perry et al., 1991b).

In a 13-week oral toxicity study, groups of 10 male and 10 female F344/N rats were fed diets containing glyphosate (purity 99%) at concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm. Ten more animals of each sex were included at each dietary concentration for evaluation of haematological and clinical pathology parameters. The calculated mean intakes were equal to 0, 205, 410, 811, 1678 and 3393 mg/kg bw per day, respectively, for males and 0, 213, 421, 844, 1690 and 3393 mg/kg bw per day, respectively, for females. All tissues from the control and highest-dose animals were examined microscopically. Salivary glands were also examined for the animals at all lower doses.

Diarrhoea was seen in males at the highest dose and in all females for the first 50 days of the study. Weight gain was reduced in males at 50 000 and 25 000 ppm, and the final mean body weight was approximately 18% and 6% less than that of controls, respectively. Small increases in several erythrocyte parameters were noted in males at 12 500 ppm and higher doses. These changes were unremarkable and generally consistent with a mild dehydration. Plasma alkaline phosphatase and alanine transaminase activities were slightly increased in males at 6250 ppm and greater and in females at 12 500 ppm and greater. In the absence of histopathological findings in the liver, these increases are considered not toxicologically significant.

No treatment-related gross abnormalities or organ-weight changes were observed at necropsy. Histopathological changes were observed only in the parotid and submandibular glands of both male and female rats. The study authors combined the findings for these two glands (Table 15). The findings for each gland individually or for individual animals were not reported. No histological alterations were observed in the sublingual gland. The changes were described as cytoplasmic alterations and consisted of basophilic changes and hypertrophy of the acinar cells. Considering the 16-fold difference between the lowest dose of 3125 ppm and the highest dose of 50 000 ppm, the incidence response curve appears to be relatively flat and the degree of change is slight, progressing from only minimal to moderate, suggesting that any changes are of equivocal toxicological significance.

Table 15. Cytoplasmic alterations of the parotid and submandibular salivary glands (combined) in rats administered glyphosate for 13 weeks

	Incidence per dietary concentration of glyphosate					
	0 ppm	**3 125 ppm**	**6 250 ppm**	**12 500 ppm**	**25 000 ppm**	**50 000 ppm**
Males	0/10	6/10 (1.0)	10/10 (1.0)	10/10 (1.8)	10/10 (2.7)	10/10 (2.9)
Females	0/10	8/10 (1.0)	10/10 (1.0)	10/10 (2.1)	10/10 (2.4)	10/10 (1.0)

ppm: parts per million

Results presented as number of rats showing cytoplasmic alterations / total number of rats in the group, with average severity score in parentheses. The severity score is based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Source: Chan & Mahler (1992)

The NOAEL in the 13-week dietary toxicity study in rats was 6250 ppm (equal to 410 mg/kg bw per day) based on the more pronounced cellular alterations in the salivary glands at 12 500 ppm and above (Chan & Mahler, 1992).

In a 90-day range-finding study, groups of 10 Sprague Dawley rats per sex were administered daily doses of glyphosate technical (purity 97.5%) at concentrations of 0, 2000, 6000 and 20 000 ppm

(equal 0, 125.2, 371.9 and 1262.1 mg/kg bw per day for males and 0, 156.3, 481.2 and 1686.5 mg/kg bw per day for females) in the diet. Blood was collected pretreatment and at termination to measure selected haematological and clinical chemistry parameters. At necropsy, selected organs were weighed. Histopathological examination was conducted on all tissues taken at necropsy.

Diets were homogeneously distributed and stable for at least 10 days. Analytical concentrations were within 10% of the nominal concentrations. No treatment-related effects was observed on mortality, body weights, body-weight gains, feed consumption, urine analysis, haematology and clinical chemistry parameters, ophthalmoscopic examination, organ weights and macroscopic and microscopic examinations. The only obvious treatment-related clinical observations was diarrhoea seen in all 10 males and nine females in the 20 000 ppm treatment group.

The NOAEL in the 90-day toxicity study in rats was 6000 ppm (equal to 371.9 mg/kg bw per day) based on diarrhoea at the lowest-observed-adverse-effect level (LOAEL) of 20 000 ppm (Parker, 1993).

In a 13-week feeding study, groups of 12 Sprague Dawley rats per sex were administered daily dietary doses of glyphosate (purity 95.3 %) at concentrations of 0, 3000, 10 000 and 30 000 ppm (equal to 0, 168.4, 569 and 1735 mg/kg bw per day for males and 0, 195.2, 637 and 1892 mg/kg bw per day for females) in the diet.

There were no treatment-related mortalities or clinical signs of toxicity. At 30 000 ppm, body weights were slightly lower (by about −5 to −10% in males and −5% in females) than those in the control. The overall feed consumption by males and females was comparable to the control. No treatment-related ocular effects or changes in haematological and clinical chemistry parameters were observed. At 30 000 ppm, urine pH in males and females was significantly lower ($P < 0.01$) than that in the control. Urine protein was significantly decreased ($P < 0.05$) in males and showed a decreasing trend in females. In addition, females showed a significantly ($P < 0.05$) higher urine volume, but males showed a decreasing trend in urine volume compared with the controls. At 10 000 ppm, urine, pH and protein in males were lower than those in the controls. In females, no statistically significant changes were observed in any parameter. No statistically significant changes were observed in either sex at 3000 ppm.

At 30 000 ppm, both sexes showed significant ($P < 0.01$) increases in absolute and relative weights of the caecum (with contents). In addition, females in this highest-dose group also showed significant ($P < 0.05$) increases in relative weights of the brain and liver. At 10 000 ppm, the absolute and relative weight of the caecum showed a statistically significant ($P < 0.01$) increase in males and increasing trend in females. At 3000 ppm, there were no treatment-related abnormalities in either sex (Table 16).

385

Table 16. Caecum weights of rats administered glyphosate for 13 weeks

	Absolute and relative weight per dietary concentration of glyphosate			
	0 ppm	3 000 ppm	10 000 ppm	30 000 ppm
Males				
Absolute weight ± SD (mg)	2 823 ± 794	3 187 ± 609	3 383 ± 1 081 (11%)	5 854 ± 2 053**
Relative weight ± SD (%)	0.55 ± 0.16	0.62 ± 0.13	0.64 ± 0.20 (11%)	1.22 ± 0.41**
Females				
Absolute weight in mg ± SD	2 367 ± 582	2 586 ± 462	3 546 ± 959*	5 268 ± 1 189**
Relative weight ± SD (%)	0.79 ± 0.17	0.84 ± 0.17	1.22 ± 0.32*	1.92 ± 0.41**

ppm: parts per million; SD: standard deviation; *: $P < 0.05$; **: $P < 0.01$

Relative weight = (organ weight/body weight) × 100.

Results expressed as absolute weight or relative weight and, in parentheses, this weight as a percentage of that of controls for males only.

Source: Kinoshita (1995)

At 30 000 ppm, 9 of the 12 males and 7 of the 12 females had statistically significantly distended caeca ($P = 0.01$). At 10 000 ppm, 3 of the 12 males showed distension of the caecum, but there were no macroscopic abnormalities in females. At 3000 ppm, there were no macroscopic abnormalities attributable to the treatment in either sex.

Although histopathological examinations revealed various histological changes in each treatment group of both sexes, treatment-related changes were not observed. One male at 10 000 ppm and one female at 30 000 ppm had renal lesions (polycystic kidney) and hepatic lesions (bile ductal proliferation and cholangiectasis). However, these were considered of a genetic nature and not treatment related.

The NOAEL in this 90-day toxicity study in rats was 3000 ppm (equal to 168.4 mg/kg bw per day) based on increased caecum weight at 10 000 ppm and above (Kinoshita, 1995).

In a 90-day oral toxicity study, groups of 12 male and 12 female Alpk:AP Wistar-derived rats were fed diets containing glyphosate (purity 97.4%) at concentrations of 0, 1000, 5000 or 20 000 ppm (equal to mean intakes of 0, 81, 414 and 693 mg/kg bw per day for males and 90, 447 and 1821 mg/kg bw per day for females).

There were no mortalities. A low incidence of diarrhoea and light-coloured faeces was seen in both sexes at 20 000 ppm in the second week of the study. Males at the highest dose showed statistically significant reductions in body-weight gain and food utilization efficiency compared with controls. There was some evidence for a reduction in platelet count in males and females at 5000 and 20 000 ppm. A marginal dose-related increase in prothrombin time was observed in males at all doses. The differences, however, were small and considered not of haematological significance. Plasma alkaline phosphatase and alanine transaminase activities were increased in both sexes at 20 000 ppm and, to a lesser extent, in males at 5000 ppm. In addition, plasma aspartate aminotransferase activity was increased in females at the highest dose at this early time point, but not at study termination. The changes in clinical chemistry parameters were small, often lacking a clear dose–response relationship, and therefore not considered biologically relevant. There were no treatment-related effects on urine biochemistry and organ weights.

The only notable histopathological finding was a uterine leiomyosarcoma in a female at 5000 ppm. Although these are rare, finding such a tumour in an animal at the intermediate dose was considered incidental to treatment.

The NOAEL in the 90-day toxicity study in rats was 5000 ppm (equal to 414 mg/kg bw per day) based on the reduced growth in males at 20 000 ppm (Botham, 1996).

386

In a 90-day feeding study, groups of 10 Sprague Dawley rats per sex were administered daily doses of glyphosate (purity 95.3%) at concentrations of 0, 1000, 10 000 or 50 000 ppm (equal to 0, 79, 730 and 3706 mg/kg bw per day for males and 0, 90, 844 and 4188 mg/kg bw per day for females) in the diet.

There were no deaths. Animals of both sexes treated with 50 000 ppm had soft faeces and diarrhoea throughout the study period from day 4. Both sexes at 50 000 ppm showed a reduction in body-weight gain over the first 4 weeks of treatment. Body-weight development was unaffected at the other doses. Both males and females at 50 000 ppm showed a reduction in dietary intake and feed efficiency over the first 4 weeks of treatment compared with controls. Water consumption, measured ocular parameters or haematological parameters for either sex were unaffected. Both males and females at 10 000 or 50 000 ppm showed a statistically significant ($P < 0.05$ at 10 000 ppm and $P < 0.01$ at 50 000 ppm) reduction in plasma calcium concentration and an increase in alkaline phosphatase compared with controls. A statistically significant ($P < 0.05$) increase in inorganic phosphorus and reduction in plasma creatinine were also evident in males and females at 50 000 ppm, while females at this dose level showed statistically significant ($P < 0.01$) reductions in total plasma protein and albumin compared with controls. There were no other treatment-related effects. Both males and females at 50 000 ppm showed statistically significant increases in relative liver and kidney weights compared with controls (Table 17).

Table 17. Group mean relative organ-weights of rats administered glyphosate for 90 days

Dietary concentration of glyphosate (ppm)	Mean relative organ weight (%)			
	Liver		Kidney	
	Male	Female	Male	Female
0	2.974 9 ± 0.2629	2.973 4 ± 0.1558	0.586 1 ± 0.0575	0.651 6 ± 0.0523
1 000	2.886 8 ± 0.2552	2.909 3 ± 0.2146	0.590 1 ± 0.0804	0.6257 ± 0.0375
10 000	2.885 3 ± 0.3758	2.980 1 ± 0.1556	0.607 0 ± 0.0552	0.645 4 ± 0.0532
50 000	3.243 3 ± 0.2452*	3.198 9 ± 0.2098*	0.6963 ± 0.0436**	0.718 0 ± 0.0707*

ppm: parts per million; *: $P < 0.05$; **: $P < 0.001$

Results expressed as mean organ-weight as a percentage of mean body-weight, ± standard deviation.

Source: Coles et al. (1996)

At 50 000 ppm all animals had enlarged and fluid-filled caeca while one female had gaseous distension of the stomach at the final termination. There were no treatment-related macroscopic abnormalities at 10 000 or 1000 ppm.

Treatment-related changes were observed in the caeca. Atrophy, characterized by flattening of the intestinal mucosa, was observed in five rats of both sexes at 50 000 ppm ($P < 0.05$ for male rats) and for one male and two female rats at 10 000 ppm. The etiology of this change is uncertain and may represent no more than atrophy of the mucosa resulting from caecal distension. There were no other treatment-related changes.

The NOAEL in this 90-day toxicity study in rats was 1000 ppm (equal to 79 mg/kg bw per day) based on the reduced plasma calcium concentration and increased alkaline phosphatase concentrations at 10 000 ppm (Coles et al., 1996).

Dogs

In a 7-day oral toxicity study, one male and one female beagle dog were fed gelatin capsules containing glyphosate (purity 99.5%) at increasing daily doses of 100, 300 or 1000 mg/kg bw per day. A second pair of dogs were administered gelatin capsules containing glyphosate at a dose of 1000 mg/kg bw per day for 14 consecutive days.

In the first pair of dogs, no treatment-related clinical signs or effects on body weight, body-weight gain, feed consumption and haematological parameters were observed. There was a slight increase in plasma alanine transaminase activity in the male dog, and cholesterol concentrations were slightly reduced in both the male and female. At termination, there were no treatment-attributable lesions.

In the second pair of dogs, no treatment-related clinical signs or effects on body weight, body-weight gain, feed consumption and haematological parameters were observed. Plasma alanine transaminase activity was slightly increased in the male dog which also had loose faeces throughout the study. No treatment-attributable lesions were found at termination (Goburdhun & Oshodi, 1989).

Glyphosate technical (purity 94.61%) was continuously fed in the basal diet to groups of four males and four females beagle dogs for at least 90 days. Dietary concentrations were 0, 1600, 8000 and 40 000 ppm (equal to 0, 39.7, 198 and 1015 mg/kg bw per day for males and 0, 39.8, 201 and 1014 mg/kg bw per day for females).

There were no treatment-related effects on mortality, clinical signs, body weight, feed consumption, test material intake, ocular changes or macroscopic findings.

Although statistically significant changes in haematology parameters and in some clinical chemistry parameters were observed in both sexes, these were not dose dependent. At 40 000 ppm, three females showed a decrease in urine pH at week 13, although these differences were not statistically significant. Although a statistically significant increase was noted in the relative weight of the adrenals in females at 1600 ppm, the change was considered incidental due to the lack of dose dependency. There were no histopathological changes related to the treatment in the treated groups of either sex. One female in the 40 000 ppm group showed cutaneous histiocytoma which is a nonspecific lesion in young dogs.

The NOAEL in this 90-day toxicity study in dogs was 40 000 ppm, equal to 1015 mg/kg bw per day, the highest dose tested (Yoshida, 1996).

Glyphosate acid (purity 99.1%) was administered at doses of 0, 2000, 10 000 or 50 000 ppm (equal to 0, 68, 323, 1680 mg/kg bw per day for males and 0, 68, 334, 1750 mg/kg bw per day for females) via the diet for 90 days to one control and three treatment groups each with four male and four female beagle dogs.

There was neither any mortality nor any treatment-related clinical signs of toxicity. The body-weight gain of males at the highest dose showed a slight depression throughout the study, but the differences were not statistically significant. Females at 50 000 ppm showed occasionally statistically significant slight depressions in body-weight gains throughout the study. No treatment-related ophthalmological and haematological findings or differences in urine clinical chemistry parameters and urinary sediment examinations were observed. Changes in clinical chemistry parameters were small and therefore not considered biologically relevant. Kidney weights of males at 10 000 or 50 000 ppm were slightly but not dose dependently increased. There was also a small increase in liver weight at these doses, but in male dogs only. No macroscopic or microscopic findings were observed.

The NOAEL in this 90-day toxicity study in dogs was 10 000 ppm (equal to 323 mg/kg bw per day) based on the decreased body-weight gains in female dogs at 50 000 ppm (Hodge, 1996).

143

In a 90-day feeding study, groups of four beagle dogs per sex were administered glyphosate technical (purity > 95%) at daily doses of 0, 200, 2000 and 10 000 ppm in the diet (corresponding to 0, 5.3, 53.5 and 252.6 mg/kg bw per day).

All the animals survived until scheduled necropsy. Neither clinical signs of toxicity nor treatment-related effects on body weights, urine analysis, organ weights, gross pathology or histopathology were observed.

A significant increase in clotting time and gamma-glutamyltransferase activity was observed in both sexes at the 45-day interim bleed; however, in the absence of any corresponding changes at terminal bleed or any histopathological correlate in the liver, this observation is considered to reflect a systemic error rather than a real effect. Total bilirubin was higher; however, in the absence of a histopathological correlate on the liver, the effect was not considered adverse.

The NOAEL in this 90-day toxicity study in dogs was 10 000 ppm (equal to 252.6 mg/kg bw per day), the highest dose tested (Prakash, 1999).

In a 13-week oral toxicity study, groups of four beagle dogs per sex were administered glyphosate (purity 95.7%) in daily doses of 0, 30, 300 and 1000 mg/kg bw by capsule.

One male and one female at 1000 mg/kg bw per day were euthanized in extremis; one male that vomited once in week 7 (before dosing) and had liquid faeces frequently in weeks 8 and 9 was euthanized on day 61. One female was euthanized on day 72; this animal had frequent liquid or soft faeces from week 4, was seen to vomit in week 10, and was dehydrated from week 9.

No treatment-related clinical signs were noted in the control animals or those at 30 or 300 mg/kg bw per day. The following treatment-related clinical signs were reported in animals at 1000 mg/kg bw per day (excluding those terminated in extremis, which are discussed separately): liquid or soft faeces on several occasions in all animals; vomiting in two of the three surviving females within 30 minutes or 3–5 hours after treatment; thin appearance in one of the three surviving males and all the females; dehydration in one of the three males and two of the three females; pale ears and mouth in one of the three females.

At 30 or 300 mg/kg bw per day, there were no histopathological changes or changes in the mean body-weight gain. At 1000 mg/kg bw per day, mean body-weight gain in males was slight (+4% vs +31% in controls) while females lost weight (–7% vs +14% in controls) from day 1. This effect on body weight was considered treatment-related. Feed consumption was reduced to 25–75% of the amount given. Neither ophthalmological findings nor treatment-related effects on haematological and clinical chemistry parameters were observed in any of the treated groups. Urinalysis showed a decrease in mean specific gravity in one of the three remaining males and all three remaining females at the highest dose in week 11. Mean absolute and relative prostate weights were reduced by 68% and 56%, respectively, but there were no other treatment-related effects on organ weights. All the macroscopic changes noted in surviving animals at termination were considered normal variations, except for the reduced uterus size.

The treatment-related changes in surviving animals at 1000 mg/kg bw per day consisted of increased number of adipocytes in the sternum of two of the three males and the three females, prostate atrophy in two of the three males and uterine atrophy in two of the three females.

The NOAEL in this 90-day toxicity study in dogs was 300 mg/kg bw per day for mortality and decreased body-weight gains at 1000 mg/kg bw per day (Gaou, 2007). This study found very pronounced toxic effects, results which differ considerably from what was seen in other studies in dogs or other species.

In a 52-week oral toxicity study, groups of six male and six female beagle dogs were fed gelatin capsules containing glyphosate (purity 96.13%) at a dose of 0, 20, 100 or 500 mg/kg bw per day once daily.

All the dogs survived. There were no treatment-related effects on body or organ weights or feed consumption and no clinical signs of toxicity, ocular abnormalities or changes in haematological or urinary parameters or macroscopic and histological findings.

The NOAEL in this 1-year toxicity study in dogs was 500 mg/kg bw per day, the highest dose tested (Reyna & Ruecker, 1985).

In a 1-year oral toxicity study, groups of four male and four female beagle dogs were administered gelatin capsules containing glyphosate (purity 98.6–99.5%) at concentrations of 0, 30, 300 or 1000 mg/kg bw per day once daily for 52 weeks.

There were no mortalities throughout the test period. Changes in faecal consistency (soft/loose/liquid) were recorded frequently for the highest-dose animals, starting 4 to 6 hours after dosing; these were also noted on occasion in a few animals at 300 mg/kg bw and were considered to be treatment related. Feed consumption was maximal or near maximal for all test groups. Mean body-weight gain showed a non-statistically significant reduction in males at all doses (approximately 83%, 75% and 75% that of the control group for the lowest, intermediate and highest doses, respectively) and in females at the highest dose (81% that of the control group). Ophthalmoscopic and laboratory examinations revealed no treatment-related abnormalities. Plasma glyphosate concentrations, which remained constant throughout the study, suggested that absorption was dose related; mean values detected were 0.36, 1.82 and 6.08 µg/mL for the lowest, intermediate and highest doses, respectively. At necropsy, no abnormal gross findings and no significant intergroup organ-weight differences attributable to treatment with glyphosate were noted. In males, absolute and relative weights of the liver were slightly but nonsignificantly increased (4%, 8% and 10% above that of the control group for absolute weights, and 10%, 17% and 19% above that of the control group for relative weights for the groups for the lowest, intermediate and highest doses, respectively). There were no significant histopathological findings at any dose.

The NOAEL in this 52-week study in dogs was 300 mg/kg bw per day based on the changes in faecal consistency (Goburdhun, 1991).

In a 52-week oral toxicity study, groups of four male and four female beagle dogs were fed diets containing glyphosate (purity 95.6%) at concentrations of 0, 3000, 15 000 or 30 000 ppm (equal to 0, 91, 440 and 907 mg/kg bw per day for males and 0, 92, 448 and 926 mg/kg bw per day for females) for 1 year. Selected organs were weighed and specified tissues taken from all groups for histopathological examination.

There were no mortalities during the study. There was no effect on feed consumption; only three dogs left small amounts of feed intermittently during the study. Body weight was slightly reduced in females at 30 000 ppm, with a maximum reduction of 11% (compared with that of controls) in week 51. These dogs showed a gradual reduction in growth rate which was consistently significant from week 23 onwards. A similar change in body-weight gain in females at the lowest dose, although occasionally reaching statistical significance, was not regarded as treatment related since a dose–response relationship was lacking. There was no effect on body weight in males at any dose tested. There were no toxicologically significant effects on any of the haematological and clinical chemistry parameters measured or any of the clinical chemical parameters measured in urine. No adverse effects of glyphosate were seen at necropsy, and there were no treatment-related effects on organ weights. No histopathological changes attributable to administration of glyphosate were found.

The NOAEL in this 1-tear toxicity study was 15 000 ppm (equal to 448 mg/kg bw per day) based on the reduced body weights at 30 000 ppm in female dogs (Brammer, 1996).

In an 12-month oral toxicity study, groups of four male and four female beagle dogs were administered glyphosate technical (purity 94.61%) in the diet at concentrations of 0, 1600, 8000 or 50 000 ppm (equal to 0, 34.1, 182 and 1203 mg/kg bw per day for males and 0, 37.1, 184 and 1259 mg/kg bw per day for females, respectively) for 1 year. A detailed histopathological examination was performed on all sampled tissues of all dogs, except for the femur, larynx, oviducts, tongue, ureter and vagina.

There were no deaths in any dose groups of either sex. No treatment-related effects were observed during periodic clinical and eye examinations, in urine analysis, weight change and macroscopic and histopathological findings. At 50 000 ppm, three of the four males and four of the four females had loose stools. The animals in the 8000 and 1600 ppm groups did not show any clinical signs. At the end of the study, mean body weights at 50 000 were reduced by 6% in males and 11% in females compared to the controls, but these reductions were not statistically significant. Feed consumption was unaffected.

Males showed no significant changes in any haematological parameters. Females at 50 000 ppm had significantly decreased haematocrit, haemoglobin concentrations and erythrocyte count. However, these changes were small and often lacked a dose–response, and so were not considered biologically relevant.

Females at 50 000 ppm showed significant changes in clinical chemistry parameters. However, these changes were within biological variability ranges and therefore not considered adverse.

The NOAEL in this 12-month toxicity study was 8000 ppm (equal to 182 mg/kg bw per day based on the loose stools in both sexes and decreased body weights in females at 50 000 ppm (Nakashima, 1997).

In a 12-month oral toxicity study, groups of four beagle dogs per sex were administered 0, 30, 100 and 300 mg/kg bw per day glyphosate technical (purity 97.5%) daily in gelatin capsules. Dose formulations were prepared weekly by adding the required amount to the capsules.

No deaths occurred in any group. At the highest dose, all males and females had soft stools, diarrhoea or mucous faeces and, rarely, bloody stools or faeces visibly containing the test material as well as vomiting. At 100 mg/kg bw per day, changes were similar to those observed at 300 mg/kg but at lower frequencies. A histopathological examination of a mid-dose male with bloody faeces continually from day 346 onward showed an ulcer by intussusception. Changes observed at 30 mg/kg bw per day were comparable to those observed in untreated animals. A significant decrease in body weight compared to that of the control group was recorded from week 24 in females at 300 mg/kg ($P < 0.01$) and from week 27 in females at 30 mg/kg ($P < 0.05$) largely continually until the end of the administration period. There were no treatment-related effects in females at 100 mg/kg. There were no treatment-related changes in feed consumption, urine analysis, haematology, blood biochemistry, ophthalmoscopy, organ weights, necropsy or histopathology.

The NOAEL was 30 mg/kg bw per day based on the changes in faecal consistency in male and female dogs and reduced body weights in females at the LOAEL of 100 mg/kg bw per day group (Teramoto, 1998).

In a 1-year oral toxicity study, groups of four beagle dogs per sex were administered glyphosate technical (purity 95.7%) at daily doses of 0, 30, 125 and 500 mg/kg bw per day in gelatin capsules for 52 consecutive weeks.

No mortalities occurred during treatment. There were no treatment-related effects on clinical signs, body weight, feed consumptions, haematology and clinical chemistry parameters, ophthalmoscopic findings, organ weights, macroscopic or microscopic findings.

The NOAEL in this 1-year toxicity study in dogs was 500 mg/kg bw per day, the highest dose tested (Haag, 2008).

(b) Dermal application

Rats

In a 21-day dermal toxicity study, groups of five male and five female Alpk:APfSD rats were exposed to glyphosate (purity 95.6%) at 0, 250, 500 or 1000 mg/kg bw per day. The test material was moistened with deionized water and the resultant paste spread on the previously clipped back of each of the animals on a gauze patch that was covered with occlusive dressing. The application site was rinsed after 6 hours of exposure. A total of 15 six-hour applications were made over 21 days.

No treatment-related effects were noted on mortality, body or organ weights, body-weight gains, feed consumption, haematology, clinical chemistry parameters, macroscopic findings and histopathological findings at any doses.

The systemic toxicity NOAEL in this 21-day dermal toxicity study in rats was 1000 mg/kg bw per day, the highest dose tested (Pinto, 1996).

Rabbits

In a 21-day GLP-compliant dermal toxicity study, groups of 10 male and 10 female New Zealand White rabbits were exposed to glyphosate (purity not reported) at 0, 100, 1000 or 5000 mg/kg bw per day. The test material was moistened with physiological saline and applied onto the skin, which was then covered with a gauze patch secured with a tape. The material was applied on intact skin (5/sex per dose) and abraded skin (5/sex per dose) for 6 hours per day, 5 days per week, for 3 weeks. Physiological saline only was applied onto the control group.

There were no deaths and no clear effects on clinical condition. Slight dermal irritation was noted in both intact and abraded skin at 5000 mg/kg bw per day but not at milder doses or the control. No treatment-related effects were observed on body weights, body-weight gains, feed consumption, haematology and clinical chemistry parameters at any doses. At termination, no treatment-related macroscopic lesions were observed at the application site or in any other tissues or organs from all test groups. No treatment-related variations in organ-weight or histopathological findings were noted.

The systemic toxicity NOAEL in the 21-day dermal toxicity study in rabbits, was 5000 mg/kg bw per day, the highest dose tested (Johnson, 1982).

In a 28-day dermal toxicity study, groups of five male and five female New Zealand White rabbits were exposed to glyphosate (purity 99.6%) at 0, 500, 1000 or 2000 mg/kg bw per day. The test material was homogenized in water, placed on a gauze pad and then applied to the clipped area of rabbit skin. The pad was covered with a sheet of polyethylene material secured with tape. The test material covered approximately 10% of the body surface area.

No treatment-related effects were noted on mortality, body weights, body-weight gains, feed consumption, haematology, clinical chemistry parameters, macroscopic findings, organ weights and histopathological findings at any dose. Very slight erythema was observed in 2000 mg/kg bw per day dose group.

The systemic toxicity NOAEL in the 28-day dermal toxicity study in rabbits was 2000 mg/kg bw per day, the highest dose tested (Tornai, 1994).

392

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In an unpublished non-GLP carcinogenicity study, glyphosate (purity 99.7%) was administered in the diet to groups of 50 male and 50 female CD-1 mice per dose at concentrations of 0, 1000, 5000 or 30 000 ppm (equal to 0, 157, 814, 4841 mg/kg bw per day, respectively, for males and 0, 190, 955, and 5874 mg/kg bw per day, respectively, for females) for 24 months. Cage-side and detailed clinical observations were conducted and body weight and feed intake monitored throughout the study. Water consumption was measured during months 12 and 24. Erythrocyte, as well as total white blood cell counts and differentials, were conducted at months 12, 18 and 24. Tissues and organs were collected from all mice whether they died during the study or were terminated. Microscopic analyses were conducted on all collected tissues.

Analysis of treated diets demonstrated that glyphosate homogeneously mixed with rodent diet remained stable for the 1-week feeding period used in this study. Glyphosate test concentrations averaged approximately 95% of the target concentrations throughout the study. No treatment-related physical or behavioural signs of toxicity or mortality were observed. Yellow staining of the anogenital area, scabbing on the ears, alopecia, excessive lacrimation, displacement of the pupils and ocular opacities seen in all groups of male and female mice were not dose related; all occurred at low incidences. Body weights for both males and females at 30 000 ppm were consistently less than the controls throughout the study. Although the decreases were slight (1%–11%), several were statistically significant. Other statistically significant decreases were noted in the mid- and low-dose animals; however, these were sporadic and did not reflect a recognizable dose–response relationship. Although sporadic statistically significant effects were noted for feed consumption in treated male and female mice, none were dose or treatment related. Also, no treatment-related effects were observed for water consumption. No biologically or toxicologically relevant effects were noted on total erythrocyte or white blood cell counts, haemoglobin, haematocrit or platelet counts. No treatment-related changes were observed in absolute or relative organ weights. Several statistically significant changes in organ/body weight ratios were observed, but these were attributed to the statistically significant decreases in terminal (fasted) body weights rather than to specific organ effects. There were no dose–response relationships or any correlated gross or microscopic observations in any of the organs.

No remarkable treatment-related effects were noted at necropsy. Statistically significant positive trends were observed for central lobular hepatocyte hypertrophy, centrilobular hepatocyte necrosis (Table 18) and chronic interstitial nephritis in males, and for proximal tubule epithelial basophilia and hypertrophy in females. Statistically significant increases in the incidence of lesions were observed for centrilobular hepatocyte necrosis in high-dose males and proximal tubule epithelial basophilia and hypertrophy in high-dose females. While the incidences and/or dose–response trends of these individual microscopic kidney lesions were found to be statistically significant, they were considered part of a spectrum of lesions which, as a whole, constitute spontaneous renal disease.

Table 18. Hepatocellular lesions in mice administered glyphosate for 24 months

Lesion		Incidence per dietary concentration of glyphosate			
		0 ppm	1 000 ppm	5 000 ppm	30 000 ppm
Centrilobular hypertrophy	M	9/49 [a]	5/50	3/50	17/50
	F	0/49	5/50	1/49	1/49
Centrilobular necrosis	M	0/49[b]	2/50	2/50	10/50 [a,b]

F: female; M: male; ppm: parts per million

Results presented as number of mice showing hypertrophy or necrosis / number of mice examined.

[a] Statistically significant linear trend ($P \leq 0.01$) using the Cochran–Armitage test.

[b] Statistically significant increase compared to control ($P \leq 0.01$) using the Chi squared test.

Source: Knezevich & Hogan (1983)

Neoplastic outcomes were of the type common in mice of this age and strain. Of the tumour types observed, bronchiolar-alveoli tumours of the lungs, hepatocellular neoplasms and tumours of the lymphoreticular system, none were dose related and all were seen in all treatment groups (Table 19). Lymphoreticular tumours were more frequently observed in female mice, but the incidences were low and did not approach statistical significance (nonsignificant trend and pair wise comparison). With the possible exception of kidney tumours (renal tubular adenomas) in males, all tumour types were considered spurious and unrelated to treatment (see Table 19).

Table 19. Neoplasia in male and female mice treated with glyphosate for 24 months

	Incidence per dietary concentration of glyphosate							
	Males				Females			
Site / Neoplasia	**0 ppm[a]**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**	**0 ppm[a]**	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Lung								
Bronchiolar alveolar adenoma	5/48	9/50	9/50	9/50	10/49	9/50	10/49	1/50
Bronchiolar alveolar adenocarcinoma	4/48	3/50	2/50	1/50	1/49	3/50	4/49	4/50
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/48	4/50	3/50	1/50	–	–	–	–
Liver								
Hepatocellular adenocarcinoma	5/49	6/50	6/50	4/50	1/49	2/50	1/49	0/49
Hepatocellular carcinoma	0/49	0/50	0/50	2/50	2/49	1/50	0/49	4/49
Lymph node (mediastinal)								
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/45	2/49	1/41	2/49	–	–	–	–
Kidney								
Renal tubular adenoma	0/49	0/49	1/50	3/50	–	–	–	–
Lymphoblastic lymphosarcoma with leukaemic manifestations	1/49	3/49	2/50	2/50	–	–	–	–
Total lymphoreticular neoplasms (sum of lymphoblastic lymphosarcoma, composite lymphosarcoma and histiocytic sarcoma)	2/48	6/49	4/50	2/49	5/50	6/48	6/49	10/49

ppm: parts per million; PWG: Pathology Working Group

Results presented as number of neoplasm-bearing animals / number of animals examined.

[a] Incidence of effect in controls from the study report prior to PWG re-evaluation.

Source: (Knezevich and Hogan, 1983)

At the request of the USEPA, the Pathology Working Group (PWG) examined all sections of the kidneys from this study as well as additional renal sections. The PWG evaluation included a renal tubule adenoma in one control male mouse that was identified during a re-evaluation of the original renal section. The PWG noted that because differentiation between tubular-cell adenoma and tubular-cell carcinoma is not always clearly apparent and because both lesions are derived from the same cell type, it appropriate to combine the incidences for statistical analysis. Statistical analyses performed by the PWG are presented in Table 20. The PWG concluded that these lesions are not treatment-related based on the following considerations: 1) renal tubular-cell tumours are spontaneous lesions for which there is a paucity of historical control data for this mouse stock; 2) there was no statistical significance

in a pairwise comparison of treated groups with the controls and there was no evidence of a significant linear trend; 3) multiple renal tumours were not found in any animal; and 4) treatment-related nephrotoxic lesions, including pre-neoplastic changes, were not present in male mice in this study. In addition, there was no increase in non-neoplastic renal tubular lesions in male mice (e.g. tubular necrosis/regeneration, hyperplasia or hypertrophy). Although the incidence of tubular adenomas exceeded the testing laboratory's historical control range (0–3.3%), the increase at the high dose was not statistically significant compared to the concurrent controls. However, the re-analysis of the tumour indicated that kidney adenomas and kidney adenoma/carcinoma combined showed statistically significant positive trend.

Table 20. Results of re-examination of incidence of renal tumours in male mice treated with glyphosate for 24 months

Tumour type	Incidence of renal tumours per dietary concentration of glyphosate			
	0 ppm	**1 000 ppm**	**5 000 ppm**	**30 000 ppm**
Adenomas	1/49 (2%)	0/49 (0%)	0/50 (0%)	1/45 (2%)
	$P = 0.442\ 2$	$P = 1.000\ 0$	$P = 1.000\ 00$	$P = 0.757\ 6$
Carcinomas	0/49 (0%)	0/49 (0%)	1/50 (2%)	2/50 (4%)
	$P = 0.063\ 5$	$P = 1.000\ 0$	$P = 0.505\ 1$	$P = 0.252\ 5$
Combined	1/49 (2%)	0/49 (0%)	1/50 (2%)	3/50 (6%)
	$P = 0.064\ 8$	$P = 1.000\ 0$	$P = 0.757\ 6$	$P = 0.316\ 3$

ppm: parts per million

Results presented as the number of tumour-bearing animals / number of animals examined, with the resulting percentage in parentheses.

P values determined using the Cochran–Armitage test and Fisher Exact test.

Source: Knezevich & Hogan (1983)

The NOAEL for the systemic toxicity in the two-stage carcinogenicity study in mice was 5000 ppm (equal to 814 mg/kg bw per day) based on the slightly reduced body weights, increased centrilobular hepatocellular necrosis in high-dose males and proximal tubular epithelial basophilia in high-dose females seen at the systemic LOAEL of 30 000 ppm; equal to 4841 mg/kg bw per day for males and 5874 mg/kg bw per day for females (Knezevich & Hogan, 1983).

The present Meeting concluded that there is some indication, by trend test but not pairwise comparison, of induction of kidney adenomas in male mice.

In a 22-month carcinogenicity study, trimethylsulfonium carboxymethylamino-methylphosphonate (Company code SC-0224; glyphosate trimethylsulfonium; purity 56.17%) was administered in the diet to groups of 80 ICR(Crl:CD-1)BR mice per sex per dose at concentrations of 100, 1000 or 8000 ppm for 22 months (mean test material intake 11.7, 118 and 991 mg/kg bw per day for male mice and 16.0, 159 and 1341 mg/kg bw per day for female mice, respectively). One control group of 60 male and female mice were fed the basal diet only. An additional control group of 80 male and female mice were fed the basal diet plus 1% propylene glycol vehicle. Interim terminations of different numbers of mice occurred at 6, 12 and 18 months. The number of mice scheduled for the full 22-month study was 50/sex per dose. Blood samples were drawn from 10 fasted male and female mice per dose at 6, 12, 18 and 22 months for haematology and clinical chemistry measurements. At the same time points, brain cholinesterase concentrations from left and right sides of the brains of five mice/sex per dose were measured; urine analysis for 10 fasted mice/sex per dose was performed; and ophthalmoscopic examinations of all the mice were conducted. Macroscopic examinations of all the animals and histopathological examinations of selected tissues from all the animals were conducted. Selected organs were weighed.

150

The mean body weights of the highest-dose male mice were decreased by 3–11% and that of the highest-dose female mice were decreased by 4–17% during most of the study. Feed consumption was also slightly decreased in male and female mice at 8000 ppm. Survival of male mice was not affected by the treatment and the survival of female mice was apparently increased. There were no treatment-related effects on clinical signs, urine analysis, haematology and clinical chemistry parameters or ophthalmoscopic parameters at 6, 12, 18 or 22 months. Similarly, there were no treatment-related effects on organ weights (absolute or relative to body weight) and palpable masses. Analysis of the brain, erythrocytes and serum cholinesterase activity did not reveal any toxicologically significant differences. In female mice, the increased incidence of non-neoplastic epithelial hyperplasia of the duodenum at 8000 ppm was considered treatment related: the per cent response of hyperplasia in females was 10, 13, 16, 15 and 24% at 0, 100, 1000 and 8000 ppm, respectively. Male mice exhibited a treatment-related increased incidence of white matter degeneration in the lumbar region of the spinal cord at 8000 ppm. Increased white masses in male mice were 2%, 3%, 4% and 8% at 0, 100, 1000 and 8000 ppm, respectively. There were no treatment-related neoplastic lesions in male and female mice. In addition, there was no decrease in latency.

The systemic toxicity NOAEL in the 22-month carcinogenicity study in mice was 1000 ppm (equal to 118 mg/kg bw per day) based on the decreased body weights and feed consumption in both sexes and increased incidence of white matter degeneration in the lumbar region of the spinal cord in male mice and epithelial hyperplasia of the duodenum in female mice at 8000 ppm. There were no treatment-related neoplastic lesions in male and female mice (Pavkov & Turnier, 1987).

Groups of 25 male and 25 female Balb/c inbred albino mice (source not specified; 5–8 weeks old at the start of treatment) per dose were administered glyphosate technical (batch and purity not given) for 80 weeks at dietary levels of 0, 75, 150 and 300 ppm. The actual mean daily compound intake was not calculated.

Survival was not affected by treatment, and there were no overt clinical signs of toxicity. Body weight in high-dose male animals tended to decrease towards the end of treatment. In females, a similar trend was obvious from the beginning of the study up to week 21 at the highest and the mid-dose level; during the last 20 weeks, mean body weight was reduced again but only in females at the highest dose. Feed consumption was markedly diminished in high-dose males from week 9 onwards and in high-dose females from week 6. Haematology and clinical chemistry assessments showed no treatment-related changes after 9 months or after 18 months. Mean organ weights were not affected. Gross and histopathological examination did not provide evidence of treatment-related lesions. The incidence of neoplasia was not increased. The total number of tumours was considerably low in all groups.

The NOAEL for chronic toxicity in the 80-week study in mice was 150 ppm for body weight and feed consumption changes. When the usual conversion factor of 10 is applied, this value would correspond to a daily intake of 15 mg/kg bw. A no-observed-effect level could not be established because a weak effect on body weight in mid-dose females cannot be completely excluded. In contrast, the study author concluded that toxicological effects did not occur up to the highest dietary level of 300 ppm although the reduction in body weight and feed consumption was mentioned in the study report. It should be noticed that body weight and feed intake were not affected at much higher doses in the other available long-term studies in mice. Thus, it is not likely that these effects were actually related to treatment (Bhide, 1988).

The draft assessment report concluded that the study is unacceptable for a reliable assessment of carcinogenicity because the number of animals used was too small. In addition, the highest dose level of 300 ppm is considered too low. However, the study provides supplementary information about chronic toxicity.

396

In an 18-month non-GLP carcinogenicity study, glyphosate (purity unknown[7]) was administered to groups of 50 male and female CFLP/LATI mice (bred in a facility in Godollo, Hungary; 26–30 days old at study initiation) at dietary levels of 0, 100 or 300 ppm. The actual daily intake was not calculated. The administration period was 18 months.

The mortality rate was high in all study groups: only 11, 14 and 23 males and 14, 16 and 14 females survived to the scheduled termination and pathological examination in the control, low- and high-dose groups. Because clinical signs of toxicity were lacking and the mortality rates were not dose dependant, a treatment-related effect on survival is not likely. Body weight and feed consumption were not affected. Gross and histopathological examination did not reveal treatment-related changes. The overall tumour rate was high in all study groups including the controls. However, no significant difference in tumour incidence was observed between the groups.

There was no clear evidence of adverse effects of glyphosate administration up to the highest tested dose of 300 ppm (about 30 mg/kg bw per day), considered the no-observed-effect level in this study. However, the scientific value of this experiment is limited (Vereczkey & Csanyi, 1982, revised 1992).

The draft assessment report concluded that no conclusion could be reached due to the low quality of the report. The study is unacceptable as a reliable assessment of carcinogenicity because the number of animals surviving to scheduled termination and pathological examination was too small. In addition, the highest dose of 300 ppm was insufficient for evaluating carcinogenicity since no evidence of toxicity was obtained at that dose level. However, the study can be considered a source of supplementary information with regard to chronic toxicity.

In an unpublished carcinogenicity study, glyphosate (purity 97.5–100.2%) was administered to groups of 50 CD-1 mice/sex per dose in the diet at concentrations of 0, 100, 300 or 1000 mg/kg bw per day for 104 weeks. The dietary concentrations were adjusted weekly for the first 13 weeks and every 4 weeks thereafter. No interim terminations were conducted. Mortality, body weight, body-weight gain and feed consumption were monitored throughout the study. White blood cell differential counts were conducted during weeks 52, 77 and 102. Organs were weighed and tissues collected for microscopic analyses following pre-terminal deaths or at scheduled termination.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage acceptable. There were no unscheduled deaths attributable to the administration of glyphosate. No treatment-related clinical signs of toxicity or biologically relevant or toxicologically significant effects on body weight or body-weight gain were observed during the study. Although statistically significant effects were noted, none were treatment related although test groups' responses were typically higher than those in the corresponding control mice. No treatment-related effects were noted on feed or water consumption. Ophthalmoscopic examinations, urine analysis and clinical chemistry parameters were not evaluated. Intergroup differences in differential blood counts in either sex at any of the time points tested were unremarkable. The absolute and relative to body thymus weights of male mice in the 300 and 1000 mg/kg bw per day groups were statistically significantly increased, but the increase in thymus weights was slight and lacked a dose–response. No histological correlates were found. In addition, no increase in absolute or relative thymus weights were found in female mice. The incidence of lung masses was slightly increased in high-dose male mice (control: 10/50; low dose: 13/50; mid dose: 12/50; and high dose: 18/50); however, histopathology failed to reveal adverse lung findings. No increase in lung masses was found in female mice. The occurrence of mineral deposits in the brain was significantly increased in males at the highest dose compared with the control group (13/50 vs 4/49). It should be noted that this is a common finding in this strain of mice at this age.

[7] The relevant supplement was not submitted to the Meeting Rapporteur and the manufacturer's name was not provided.

There were no statistically significant increases in the incidence of any tumours, benign and malignant, in either sex; however, the number of animals with multiple tumour types was slightly increased in the high-dose group of both sexes (males: 16/50; females: 11/50) compared to the control (males: 11/50; females: 6/50). This led to a slight increase in the total number of tumours in the high-dose group of both sexes (males: 60; females: 43) compared to the control (males: 49; females: 36).

Haemangiosarcoma in the vascular system was evident in 4/50 high-dose males, 2/50 low-dose females and 1/50 high-dose females compared to 0/50 controls. Of the high-dose mice, one had tumours in the liver and spleen; one had a tumour in the liver only; one had tumours in the liver, spleen and prostate; and one had a tumour in the spleen only. The incidence of haemangiosarcoma in males was positive in Exact trend test and nonsignificant in pairwise comparison (Table 21). In female mice, incidence of haemangiosarcoma did not achieve statistical significance.

Table 21. Haemangiosarcomas in male mice administered glyphosate for 104 weeks

	Measure per dietary dose of glyphosate			
	0 mg/kg bw per day	**100 mg/kg bw per day**	**300 mg/kg bw per day**	**1 000 mg/kg bw per day**
Haemangiosarcomas	0/47 (0%)	0/46 (0%)	0/50 (0%)	4/45 (9%)
	$P = 0.002\ 96$**	$P = 1.000\ 00$	$P = 1.000\ 00$	$P = 0.053\ 32$

bw: body weight; **: significance of trend ($P < 0.01$) denoted at control, using Fisher Exact test and Exact Trend test.

Results presented as number of tumour-bearing animals / number of animals examined less those that died before week 52, with the resulting percentage in parentheses.

Source: Atkinson et al., 1993a

Histiocytic sarcoma in the lymphoreticular/haematopoietic tissue was evident in 2/50 low- and high-dose males and 3/50 low- and intermediate-dose females and 1/50 high-dose female (none were evident in the respective controls). Due to a lack of dose relationship and statistical significance, these changes are not considered treatment related. Other tumours seen were considered typical for mice of this age and strain.

The NOAEL for systemic toxicity in the 104-week carcinogenicity study in mice was 1000 mg/kg bw per day, the highest dose tested (Atkinson et al., 1993a).

In an 18-month carcinogenicity study, glyphosate (two lots of HR-001, purity 97.56% and 94.61%) was fed in the diet to groups of 50 male and 50 female ICR(Crj:CD-1)(SPF) mice at 0, 1600, 8000 or 40 000 ppm (equal to 0, 165, 838.1 or 4348 mg/kg bw per day for males and 0, 153.2, 786.8 or 4116 mg/kg bw per day for females) for 18 months. During treatment, all animals were observed for clinical signs and changes in body weight, and feed consumption was measured. At week 21, urine analysis was carried out on 20 males from all groups. Differential leukocyte counts were determined in blood smears from 10 males and 10 females from all groups at week 52 and after 78 weeks of treatment and also in animals terminated in extremis during the treatment, as possible. At final necropsy after 78 weeks of treatment, organ weights of 10 males and 10 females were analysed to determine differential leukocyte counts. All animals of both sexes were necropsied and their histopathology examined.

At 1600 ppm, there were no treatment-related changes in either sex in any parameters. At 8000 ppm, retarded growth was observed in females with statistically significant decreases in weight at week 6 and weeks 9 to 24. No treatment-related changes were seen in males. At 40 000 ppm, the incidence of pale skin increased in males. In addition, loose stools were found in all the cages from week 21 in males and week 20 in females. Retarded growth was persistently observed during treatment, with statistically significant differences in weight from week 16 to 36 in males and from week 6 to the end of the treatment in females. These changes were associated with depressed feed

398

consumption and feed efficiency. At necropsy, the increased incidences of distension of the caecum were noted in males and females in all the animals examined, which were consistent to increases in absolute and relative weights of the caecum. However, no histopathological abnormalities were recorded in the caecum. In males, a significant increase was noted for the overall incidence of anal prolapse that corresponded with erosion/ulcer of the anus.

The incidence of lymphoma was increased in the high-dose males but lacked a clear dose–response (see Table 22). It was significant by trend test and not by pairwise comparison. In female mice, the increased incidences of lymphoma were not statistically significant (trend test and pairwise comparison). The overall incidences of lymphomas observed were well below the historical control range of 0–18% (Baldrick & Reeve, 2007). Kidney adenomas and carcinomas in male mice were slightly increased at the high dose of 40 000 ppm. The statistical significance was achieved by the trend test and not by pairwise comparison. The incidences of kidney tumours in males exceeded the historical control range. Incidence of haemangiosarcomas was statistically significantly increased in the mid and high dose according to the trend test but not in a pairwise comparison.

Table 22. Selected neoplastic findings in male and female mice administered glyphosate for 18 months

	Incidence per dietary concentration of glyphosate			
Neoplastic findings	**0 ppm**	**1 600 ppm**	**8 000 ppm**	**40 000 ppm**
Males				
Lymphoma	2/50	2/50	0/50	6/50
Kidney (adenoma/carcinoma)	0/50	0/50	0/50	2/50
Haemangiosarcoma (various organs)	1/50	0/50	0/50	0/50
Females				
Lymphoma	6/50	4/50	8/50	7/50
Kidney (adenoma/carcinoma)	0/50	0/50	0/50	0/50
Haemangiosarcoma (various organs)	0/50	0/50	3/50	5/50

No.: number; ppm: parts per million

Results presented as number of tumour-bearing animals / number of animals examined.

Source: Sugimoto (1997)

Based on these results, the NOAEL was 1600 ppm (153.2 mg/kg bw per day) and the LOAEL was 8000 ppm (838.1 mg/kg bw per day) for females based upon retarded growth with statistically significant decreases in weight at week 6 and weeks 9 to 24 (Sugimoto, 1997).

In a 78-week carcinogenicity study, glyphosate (purity 97.5%) was fed to groups of 50 male and 50 female Crj:CD-1 mice per dose at dietary concentrations of 0, 500, 5000 and 50 000 ppm (equal to 0, 67.6, 685 and 7470 mg/kg bw per day for males and 0, 93.2, 909 and 8690 mg/kg bw per day for females) for 78 weeks. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were conducted and body weight and feed intake monitored throughout the study. Differential white blood cell counts were performed at week 52, and haematological parameters evaluated at the end of the treatment. Gross pathological examinations were conducted at termination and on euthanized moribund and pre-terminally dead mice. Selected organs (brain, liver, both kidneys, both adrenal glands and both testes) were weighed. The tissue samples from control and high-dose animals and animals that died or were terminated in extremis were histopathologically examined.

Prepared diets were stable at room temperature for 4 months and the test material was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations ranged from 80–110% of the nominal concentrations. At 50 000 ppm, all the mice had loose stools throughout the treatment period, although some showed improvement as treatment continued. In the same group, nine males and eight females had treatment-related anus prolapse at week 10 or later. Other clinical signs and incidences were similar in both control and treated groups. A statistically significant difference in mortality rate in males was noted between the 50 000 ppm group and the control group at week 26 or later. Mortality in mid- and low-dose males and females at all doses was unaffected. At 50 000 ppm, body-weight gain significantly decreased or appeared to decrease throughout the treatment in males and at week 24 or later in females. No effects of treatment were observed in treated males and females in the mid and low dose at any time compared to controls. In both males and females at 50 000 ppm, feed consumption decreased compared with controls; the change was considered treatment related. No treatment-related changes were observed in haematology parameters. In the females at 50 000 ppm, the relative weights of kidneys (total) significantly increased. These changes were considered treatment related, though no corresponding histopathological findings were observed. In addition, decreases in the absolute weights of liver and right and left kidneys and significant increases in the relative weights of brain, left kidney, left adrenal gland, and right and left testes in males, and a decrease in the absolute weight of brain in females were noted at 50 000 ppm. The changes in the adrenal and brain were not considered adverse since they were not accompanied with histopathological findings. Macroscopic examination revealed luminal dilation of the large intestine, which may be associated with loose stool, in most of the terminated males and females at 50 000 ppm. Treatment-related non-neoplastic lesions were found in the kidneys in males and the rectums in males and females at 50 000 ppm. The renal findings included significant increases in tubular epithelial cell hypertrophy, tubular dilation, degeneration/necrosis and an increasing tendency in basophilic tubules proliferation (based on data from all animals). The rectal findings included significant increases in anus prolapse-associated erosion and luminal dilation (Table 23).

Table 23. Non-neoplastic lesions in mice administered glyphosate for 78 weeks

	Incidence per dietary concentration of glyphosate							
	Male				Female			
Non-neoplastic lesion	0 ppm	500 ppm	5 000 ppm	50 000 ppm	0 ppm	500 ppm	5 000 ppm	50 000 ppm
Kidney								
Tubular dilation	4/50	7/50	4/50	20**/50	8/50	12/50	5/50	8/50
Tubular epithelial cell hypertrophy	13/50	10/50	13/50	25*/50	13/50	17/50	14/50	13/50
Basophilic tubules	21/50	16/50	17/50	28/50	14/50	14/50	10/50	13/50
Tubular degeneration/necrosis	9/50	6/50	5/50	15/50	5/50	8/50	8/50	7/50
Rectum								
Luminal dilation	0/48	0/12	0/7	6*/46	0/44	0/11	0/10	6*/44
Erosion	0/48	0/12	0/7	3/46	0/44	0/11	0/10	6*/44

ppm: parts per million; *: $P < 0.05$, **: $P < 0.01$ (Fisher Exact test).

Results presented as number of tumour-bearing animals / number of animals examined.

Source: Takahashi (1999a)

Incidences of lymphomas in female mice were 3/50, 1/50, 4/50 and 6/50 in the control, 500, 5000 and 50 000 ppm dose group, respectively. The increased incidences of lymphoma at high doses were statistically significant in the trend test but not in a pairwise comparison. Renal cell adenoma was observed in three males and renal cell carcinoma in one male at 50 000 ppm; renal cell adenoma

400

was also observed in one male at 5000 ppm and none in any of the females (based on data from all animals). The incidence of other tumour types in glyphosate-treated groups and controls were similar.

These tumours were re-examined by the original study pathologist in 2012 because the Pesticide Expert Panel, Food Safety Commission of Japan requested more information on historical control data and association with the non-neoplastic renal findings. The haematoxylin-and-eosin–stained kidney sections prepared in the original study had faded and could not be evaluated; the paraffin-embedded blocks of 50 males from each group which had been stored for each observation period were sectioned and stained by haematoxylin and eosin for microscopic re-examination. The data from the re-examination and the original data are shown in Table 24.

Table 24. Renal tumours in male mice administered glyphosate for 78 weeks

Dietary concentration of glyphosate (ppm)	Findings	No. of cases		Incidence[a]
		Original study	Re-examination	
50 000	Renal cell adenoma	3	1	1/50 (2%)
	Renal cell carcinoma	1	1	1/50 (2%)
5 000	Renal cell adenoma	1	1	1/50 (2%)
500	Renal cell adenoma	0	1	1/50 (2%)

no.: number; ppm: parts per million

[a] Results presented as number of tumour-bearing animals / number of animals examined, with the resulting percentage in parentheses.

Source: Nippon Experimental Medical Research Institute (2012)

Upon re-examination (using Fisher Exact probability test, $P > 0.05$), the incidence of renal tumours in each treatment group no longer significantly differed from that in the control group. The historical control data for the Takahashi (1999a) study were not available, but the historical control values described in the re-examination document for renal cell carcinoma were 1/725 (0.13%) in males and 0/725 (0%) in females and for renal cell adenoma were 3/564 (0.53%) in males and 0/564 (0%) in females (Chandra & Frith, 1994; Baldrick & Reeve, 2007). The re-examination report also provides reference data: 0/55, 0/55, 1/55, 0/55 and 0/55 (0–1.8%) in males and 0/55 for all doses (0%) in females for renal cell carcinoma; and 0/55, 1/55, 1/55, 1/55, 0/55 (0–1.8%) in males and 0/55, 0/55, 0/55, 0/55, 1/55 (0–1.8%) in females for renal cell adenoma. The results of the re-examination revealed that the incidence of tubular epithelial cell hypertrophy in each treatment group did not significantly differ from that in the control group. In addition, the tubular epithelial cell hypertrophy was localized. These findings indicate no association between the tubular epithelial cell hypertrophy and the development of renal tumours.

In conclusion, the renal cell tumours observed in this study are not relevant for human risk assessment because (1) the incidence of renal tumours in males at 50 000 ppm did not significantly differ from that in the control group up on re-evaluation; (2) none of the females had neoplastic or non-neoplastic lesions; and (3) the highest dose (50 000 ppm) used in this study far exceeded the limit dose for mice (7000 ppm) specified by the Organisation for Economic Co-operation and Development (OECD) and USEPA.

The NOAEL in the 78-week carcinogenicity study in mice was 5000 ppm (equal to 685 mg/kg bw per day) for loose stools, decreased body-weight gain, decreased feed consumption and increased incidences of rectal and renal non-neoplastic lesions observed in male and female mice at the LOAEL of 50 000 ppm (equal to 7470 mg/kg bw per day), the highest dose tested (Takahashi, 1999a).

In an 18-month carcinogenicity study, glyphosate (purity > 95%) was fed to groups of HsdOla:MF1 Swiss Albino mice (50/sex per dose) in the diet at concentrations of 0, 100, 1000 or

10 000 ppm (equal to 0, 14.5, 149.7 and 1453 mg/kg bw per day for males and 0, 15.0, 151.2 and 1466.8 mg/kg bw per day for females) for 18 months. The stability, homogeneity and dietary concentrations were measured periodically. All the prepared diets were stable for 30 days. The test material was homogenously distributed; mean prepared dietary admixture concentrations were within 10% of the nominal concentration for all diet samples.

A detailed veterinary examination of all mice was conducted before and after grouping and monthly thereafter. Clinical signs of toxicity, appearance, behaviour and neurological changes and mortality of all mice were checked daily. Ophthalmological examinations of all mice occurred prior to the start of treatment and at 6, 12 and 18 months. Mortality, body weight, body-weight gain and feed consumption were monitored throughout the study. White blood cell differential counts were conducted at 9 months and at scheduled termination of all surviving animals and those terminated in extremis. All the animals that died or were terminated in extremis were necropsied immediately or preserved in 10% buffered neutral formalin until necropsy. All the surviving mice were terminated at scheduled termination. A gross pathological examination was performed on all mice. Adrenals, kidneys, liver and gall bladder, ovaries and testes from 10 mice per sex per dose were weighed, and selected tissues from control and high-dose animals and those animals that died or were terminated in extremis histopathologically examined.

There were no treatment-related effects on clinical signs, body weights, body-weight gains, feed consumption, ophthalmoscopic examination or absolute and relative organ weights. The survival percentage was slightly decreased at the highest dose, but the decrease was not statistically significant and the mortality at 10 000 ppm remained within the historical control range. There were no significant treatment-related changes in the white blood cell counts for either sex at 9 or 18 months.

In mice found dead or terminated moribund, cystic glands of the stomach were significantly increased in high-dose males and for both sexes combined, but did not show dose dependency and were considered incidental. Observations at lower doses or findings that were not dose dependent included increased haematopoiesis in femurs of high-dose males and mid- and high-dose combined sex groups; increased cell debris in tubules of epididymides in mid-dose males; increased incidence of subcapsular cell hyperplasia in the adrenals of low-dose males; decreased incidence of kidney nephropathy in mid-dose females; and decreased incidence of lymphocyte infiltration of epididymides in mid-dose males. At termination, cystic glands of the stomach were significantly increased in low-, mid- and high-dose males but without a dose–response relationship. Degenerative heart changes were higher in high-dose males and females, and significantly higher when sexes were combined, but the incidences were similar to the historical controls and the severity was not dose dependant. In mandibular lymph nodes, lymphoid hyperplasia was significantly increased in low- and mid-dose males and when sexes were combined, whereas the incidence was significantly lower in high-dose females. In addition, extramedullary haematopoiesis was significantly increased in these lymph nodes at the mid-dose level when sexes were combined. Extramedullary haematopoiesis in the spleen was significantly increased in females and when the sexes were combined at the low-dose level. In the absence of any dose relation, these findings, as well as several statistically nonsignificant changes, were considered incidental.

The number of malignant lymphoma (Table 25) was slightly elevated in the high-dose group compared to controls. However, this haemolymphoreticular system tumour is one of the most common, accounting for the highest percentage of spontaneous tumours in mice, and the observed incidence is considered incidental and not treatment related. A statistically significant increase in malignant lymphoma was noted in both the male and female high-dose groups. Although malignant lymphoma are common in mice, accounting for 54.6% of all tumours in this study, that the higher incidence in the high-dose groups is treatment related cannot be excluded.

402

Table 25. Malignant lymphoma in glyphosate-treated mice

			Measure per dietary concentration of glyphosate							
			Males				Females			
	M	F	0 ppm	100 ppm	1 000 ppm	10 000 ppm	0 ppm	100 ppm	1 000 ppm	10 000 ppm
Dead and moribund mice										
No. examined	75	77	22	20	22	27	16	16	20	20
No. affected	20	49	9	12	13	13	9	10	13	12
Incidence (%) [a]	26.7	63.6	41.0	60.0*	59.0*	48.0	56.0	63.0	65.0	60.0
Terminated mice										
No. examined	175	173	28	30	28	23	34	34	30	30
No. affected	26	50	1	3	3	6*	9	10	6	13
Incidence (%) [a]	14.9	28.9	3.6	10.0	10.7	26.1*	26.5	29.4	20.0	43.3*
Mean percentage	14.9	28.8	–	–	–	–	–	–	–	–
Range of percentage	8–24	2–43	–	–	–	–	–	–	–	–
All fates										
No. examined	250	250	50	50	50	50	50	50	50	50
No. affected	46	99	10	15	16	19*	18	20	19	25
Incidence (%) [a]	18.4	39.6	20.0	30.0	32.0	38.0*	36.0	40.0	38.0	50.0*
Mean percentage	18.4	41.6	–	–	–	–	–	–	–	–
Range percentage	6–30	14–58	–	–	–	–	–	–	–	–

F: females; M: males; –: not examined/not determined; *: significant increase compared with historical controls (no *P* value provided)

[a] Incidence expressed as number of animals affected as a percentage of the number examined.

Source: Kumar (2001)

The increased incidences of kidney tumours at high doses (0/50, 0/50, 1/50 and 2/50 at 0, 100, 1000 and 10 000 ppm, respectively) were statistically significant in the trend test but not in a pairwise comparison. No historical control data were available.

The NOAEL for systemic toxicity in the 18-month carcinogenicity study in mice was 1000 ppm (equal to 149.7 mg/kg bw per day) for increased mortality at 10 000 ppm. Glyphosate was not carcinogenic in mice at doses up to 10 000 ppm, the highest dose tested (Kumar, 2001).

In a carcinogenicity study, glyphosate (purity 95.7%) was fed in the diet to groups of 51 male and 51 female CD-1 mice per dose at concentrations of 0, 500, 1500 and 5000 ppm (equal to 0, 71.4, 234.2 and 810 mg/kg bw per day for males and 0, 97.9, 299.5 and 1081.2 mg/kg bw per day for females) for 79 weeks. An additional 12 mice per sex, designated as veterinary controls, were housed and maintained alongside the treated animals. Ten animals per sex from each group were set aside for an interim termination (toxicity assessment) at week 39. Stability, homogeneity and dietary concentrations were evaluated periodically. Cage-side and detailed clinical observations were conducted, and body weight and feed intake monitored throughout the study. Water consumption was observed daily. Blood smear samples were collected after 12 months and at termination from all animals and from mice terminated in extremis. Differential white blood cell counts were performed on all control and high-dose animals and on the animals terminated in extremis. Gross pathological examinations were conducted at termination and on moribund and pre-terminally dead mice. Selected

organs of 10 mice per sex per dose were weighed. Histopathological examination was performed on all sampled tissues from control and high-dose animals and on animals that died or were terminated in extremis.

Analyses indicated that the dose preparations were homogeneous and stable for at least six weeks and that the mean prepared dietary admixture concentrations were within 5% of the nominal concentration for all doses except one low-dose sample, which was over 10% of the nominal concentration.

There were no treatment-related effects on the number of mortalities observed and no significant differences in mortality rates during the study. No significant treatment-related clinical observations were reported. Similarly, no treatment-related effects on body weights, body-weight gains, absolute or relative organ weights, and feed and water consumption were observed. There were no significant differences in proportion of white blood cell populations of either sex at both 12 and 18 months, no trends in the proportion of palpable masses and no treatment-related macroscopic findings observed for any of the mice. There appears to be a dose-related increase in malignant lymphomas in the male mice only (0/51, 1/50, 2/51 and 5/51 at 0, 500, 1500 and 5000 ppm, respectively). The increased incidences at high doses were statistically significant in the trend test and not in a pairwise comparison; they are attributed to an unusually low incidence in the controls[8] (and presumably also for the low-dose treated mice). The observed increase appears to be well within the historical range and thus not biologically significant.

The NOAEL for carcinogenicity and systemic toxicity was 5000 ppm (equal to 810 mg/kg bw per day) in the 79-week study in mice, the highest dose tested (Wood et al., 2009a).

Roundup Original (glyphosate 41%, polyoxyethylenеamine [POEA] approximately equal to 15%) was evaluated in Swiss mice for tumour promotion via topical administration using a two-stage cancer model. In this study, a known tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), and tumour initiator, 7,12-dimethylbenz[a]anthracene, were used. Proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry showed that 22 spots were differentially expressed (> twofold) on glyphosate, 7,12-dimethylbenz[a]anthracene and TPA application compared with the untreated control. Among them, nine proteins (translation elongation factor eEF-1 α chain, carbonic anhydrase III, annexin II, calcyclin, fab fragment anti-VEGF antibody, peroxiredoxin-2, superoxide dismutase [Cu–Zn], stefin A3 and calgranulin-B) were common and showed similar expression pattern in glyphosate and TPA-treated mouse skin. The study authors concluded that this glyphosate formulation has tumour-promoting potential in skin and that its mechanism seemed similar to that of TPA (George et al., 2010).

Rats

In a non-GLP combined chronic toxicity and carcinogenicity study, groups of Sprague Dawley rats (50/sex per dose) were fed diets containing glyphosate (purity 98.7%) at concentrations of 0, 30, 100 or 300 ppm for the first week. Concentrations were subsequently adjusted so actual doses of 0, 3.05, 10.30 and 31.49 mg/kg bw per day in males and 0, 3.37, 11.22, and 34.02 mg/kg bw per day in females were maintained for approximately 26 months. The diets were periodically analysed for stability, homogeneity and dietary concentrations. All the rats were observed twice daily for mortality and toxic signs. Body weights and feed consumption were determined at pretest, weekly for 14 weeks and biweekly thereafter. Water consumption was determined for 10 rats/sex per group for two separate 3-day periods at 18 and 24 months. Blood and urine samples were collected at 4, 8, 12, 18 and 24 months from 10 rats/sex per group. Selected haematological and clinical chemistry

[8] The historical control value for lymphomas in CD-1 mice from the testing facility, Harlan laboratory, in 2000–2010 ranged from 0–32% with a mean of 7.51% (letter from Wood E to Bond A, Regulatory Affairs Manager, Nufarm UK, Ltd., titled 'Historical incidences of malignant lymphoma in CD-1 mouse').

parameters were evaluated. Complete necropsies were performed on all rats that died or were terminated during or at the end of the study. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, testes/ovaries, pituitary, spleen and thyroid. The tissues were preserved for histopathology.

There was no significant difference in survival rate between the control and treated groups of both sexes, and survival was approximately 80–90% through month 20 of the study for all groups. No treatment-related clinical observations were reported in any of the treated groups. Although statistically significant differences in mean feed consumption were occasionally noted, these differences occurred sporadically and were not dose dependant. Water consumption of the treated and control groups were similar at the 18- and 24-month intervals. During the intermediate months, mean body weights of the treated animals were slightly lower than that of the controls. Maximum body-weight reductions for males ranged from 6% in the high-dose group to 2–3% in the low-dose group. For females these differences were statistically significant only during months 20 and 21 and were not dose related. From month 24 until study termination the mean body weights of all treated groups were comparable to the controls. Haematology, blood biochemistry and urine analysis parameters deviated occasionally and some of them differed significantly from controls, but these differences were not dose related and not consistent over time or between sexes. No statistically significant differences were noted in the absolute and relative organ weights of the treated groups compared to the controls. The few intergroup differences were neither dose related nor consistent. Lesions consisting primarily of inflammatory and structural changes that are common in rats of this strain in lifetime studies were similar in incidence and severity to control groups for both sexes. The most frequently observed changes occurred in the lungs and the kidneys, and were associated with chronic respiratory disease and chronic progressive nephropathy. Both these sites of lesions (lungs and kidneys) are a common age-related disease in this strain of rats.

A variety of neoplasms were found in both control and treated animals, the most common being common spontaneous neoplasms in the pituitary glands and in the mammary glands. Female rats showed an increased incidence of spleen and liver lymphoma combined (positive trend: 0/50, 0/50, 1/50 and 2/50 at 0, 30, 100 and 300 ppm respectively); however, the pair wise comparison was nonsignificant. Similarly, pancreatic islet cell tumours were observed in male rats with no clear dose–response relationship. The incidence of all tumour-bearing animals in the treated groups and the controls were similar (19–23% combined adenomas and carcinomas for males and 36–42% for females) and did not exhibit a dose–response relationship.

The pancreatic islet cell tumours were observed in male rats with no clear dose–response relationship. The Meeting concluded that the pancreatic islet cell adenoma and carcinoma were incidental for several reasons: the tumours occurred in only one study in males only; other studies that used appreciably higher doses did not find any excess tumours; there was no dose–response relationship; and incidences in controls was unusually low; the Meeting also noted that there was a negative dose–response relationship in females.

Although the incidence of interstitial cell tumours in the testes was increased in the treated animals (12% at the highest dose at termination), this was not considered relevant to human risk assessment based on the following weight-of-evidence considerations: 1) a monotonic dose–response relationship was lacking; 2) pre-neoplastic lesions (i.e. interstitial cell hyperplasia) were absent; 3) the incidences were within the normal biological variation seen for this tumour type in this strain of rats; 4) the incidences in the concurrent controls (0%) was not representative of the normal background incidences noted in the historical control animals; and 5) no interstitial cell tumours were seen when tested at much higher doses in the same strain of rats in an another study of glyphosate (Stout & Ruecker, 1990); and 6) due to major differences between rodents and humans with respect to prevalence of different testicular tumour types, hormonal physiology and response and risk factors for Leydig cell tumours, chemical induction of Leydig cell tumours in rats is generally considered of limited relevance to humans (Alison, Capen & Prentice, 1994; Clegg et al., 1997; Cook et al., 1999).

405

The NOAEL for systemic toxicity in rats after 26 months of dietary exposure to glyphosate was 31.5 mg/kg bw per day, the highest dose tested. It was concluded that the glyphosate was not carcinogenic in rats (Lankas, 1981).

In a 24-month combined chronic toxicity and carcinogenicity study, groups of Sprague Dawley rats were fed daily dietary doses of 0 (group 0, with 60 rats/sex, was fed basal diet with no vehicle, and group 1, with 80 rats/sex, was fed the basal diet plus the propylene glycol vehicle), 100 (group 2, with 80 rats/sex), 500 (group 3, with 80 rats/sex) and 1000 (group 4: 90 rats/sex) ppm of active ingredient (0, 178, 890 and 1779 ppm technical glyphosate trimesium [trimethylsulfonium carboxymethylamino-methylphosphonate, company code SC-0224]. Average doses for the 2-year treatment period, based on the nominal concentrations of active ingredient, were 4.2, 21.2 and 41.8 mg/kg bw per day for males and 5.4, 27.0 and 55.7 mg/kg bw per day for females.

Interim terminations of between 10 and 20 rats took place at 6, 12 and 18 months. All the surviving rats in all groups were terminated at 24 months.

The only indication of toxicity was a significant reduction in growth in both sexes in group 4 (1000 ppm). The test material at the doses tested did not cause dose-related effects involving survival, histopathological changes or any indications of carcinogenicity. Although various common tumour types were found in both sexes, the majority were pituitary and mammary gland adenomas and adrenal pheochromocytomas, which occurred at comparable incidences in the controls.

The NOAEL for systemic toxicity was 500 ppm in rats (equal to 21.2 mg/kg bw per day) based on the significant reduction in growth at 1000 ppm in both sexes. There was no evidence of carcinogenicity of glyphosate trimesium in rats in this study (Pavkov & Wyand, 1987).

In a 2-year combined chronic toxicity and carcinogenicity study, groups of Sprague Dawley rats (60/sex per dose) were fed diets containing glyphosate (purity 96.5%) at dietary concentrations of 0, 2000, 8000 or 20 000 ppm for 24 months (equal to 0, 89, 362 or 940 mg/kg bw per day for males and 0, 113, 457 or 1183 mg/kg bw per day for females). All animals were observed twice daily for mortality and moribundity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and feed consumption were determined each week for the first 13 weeks and then every fourth week thereafter. Ophthalmic examinations were performed at pretest and just prior to termination. Haematology, blood biochemistry and urine analysis tests were conducted on 10 animals per sex per dose at months 6, 12 (the interim termination), 18 and 24 (study termination). Ten animals per sex per dose were terminated at month 12, and all the survivors at month 24. All animals were given a complete gross necropsy. Brain, kidneys, liver and testes with epididymides were weighed. Approximately 40 tissues were preserved and examined microscopically.

Analyses indicated that the neat test material was stable throughout the study, that the homogeneity of the diet mixtures was adequate, and that average glyphosate concentrations were 95% of target levels for all dose groups. There were no statistically significant differences in group survival rates. At the end of the study, the percentages of animals surviving at 0, 2000, 8000, and 20 000 ppm were 29%, 38%, 34% and 34% for males, respectively, and 44%, 44%, 34% and 36% for females. Various clinical signs were noted throughout the study, but they were typical of those frequently observed in chronic studies and appeared to be randomly distributed in all groups. Statistically significant reductions in body weight were noted in high-dose females from week 7 through approximately month 20. During this time, absolute body weights gradually decreased to 14% below the control value. Body-weight gain in high-dose females was also consistently reduced compared to the controls. At the point of maximum body-weight depression (20 months), cumulative body-weight gain was 23% less than control. Body-weight gain in all treated male groups was comparable to controls. No statistically significant decreases in feed consumption in either sex took place at any time in the study; significant increases were noted frequently in high-dose males.

406

The ophthalmic examination prior to study termination revealed a statistically significant difference ($P < 0.05$) in the incidence of cataractous lens changes between control and high-dose males (0/15 vs 5/20). This incidence (25%) was within the range (0–33%) observed in previous studies of untreated male CD rats at this laboratory (Monsanto Agricultural Company, St. Louis, MO, USA). The incidences of cataractous lens changes in low- and mid-dose males, as well as all treated female groups, were comparable to their respective controls. An examination by an independent pathologist from Monsanto (Dr Rubin) also showed a statistically significant increase ($P < 0.05$) in cataractous lens changes in high-dose male animals (8/19 vs 1/14 for controls) and concluded that a treatment-related occurrence of lens changes affected high-dose males. Further histopathological re-evaluation of eyes by Experimental Pathology Laboratories Incorporation revealed cataract and/or lens fibre degeneration (Table 26). Because the number of rats ophthalmologically examined and affected at termination was small, the results are difficult to interpret. Nevertheless, the occurrence of degenerative lens changes appears to be exacerbated by treatment in high-dose males.

Table 26. Cataract and lens fibre degeneration in male rats administered dietary glyphosate for 24 months

	Incidence per dietary concentrations of glyphosate			
	0 ppm	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Terminal kill	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

ppm: parts per million

Results presented as number of rats affected / number of rats examined.

Source: Strout & Ruecker (1990)

While there were various changes in haematology and serum chemistry parameters, these were not consistently noted at more than one time point; were small and within historical control ranges; and/or did not occur in a dose-related manner and so were considered either unrelated to treatment or toxicologically insignificant. There was a statistically significant increase in urine specific gravity in high-dose males at 6 months and statistically significant reductions in urine pH in high-dose males at months 6, 18 and 24 months; this may have been due to the excretion of glyphosate, which is an acid. Statistically significant increases in liver-to-body weight ratio at 12 months and absolute liver weight and liver-to-brain weight ratio at 24 months occurred in males at 20 000 ppm. There were no other statistically significant changes in organ weights. Gross abnormalities seen at necropsy were not glyphosate related.

Histopathological examination revealed an increase in the number of mid-dose females with inflammation of the stomach squamous mucosa, the only statistically significant occurrence of non-neoplastic lesions. Although the incidence (15%) of this lesion in mid-dose females was slightly outside the laboratory historical control range (0–13.3%), there was no dose-related trend across all groups of treated females and no significant difference in any male group, leading to the conclusion that the finding was not treatment related (Table 27).

Table 27. Inflammation of the stomach squamous mucosa in rats administered glyphosate for 24 months

	Incidence per dietary concentrations of glyphosate			
	0 ppm	2 000 ppm	8 000 ppm	20 000 ppm
Males	2/58	3/58	5/59	7/59
Females	0/59	3/60	9/60**	6/59

ppm: parts per million; **: $P \leq 0.01$ (Fisher Exact test with Bonferroni inequality)

Results presented as number of rats with the inflammation / number of rats examined.

Source: Strout & Ruecker (1990)

The only statistically significant difference in neoplastic lesions between control and treated animals was an increase in the number of low-dose males (14%) with pancreatic islet cell adenomas (Table 28). The historical (1983–1989) control range for this tumour at the testing laboratory was 1.8–8.5%, but a partial review of reported studies revealed a prevalence of 0–17% in control males with several values greater than or equal to 8%. The incidences of islet cell adenomas did not follow a clear dose-related trend in the treated male groups as indicated by the lack of statistical significance in the Peto trend test, meaning that the distribution of incidences in the four groups was most likely random. There was also considerable intergroup variability in the numbers of females with this tumour (5/60, 1/60, 4/60 and 0/59 in the control, low-, mid- and high-dose groups, respectively) and no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a control male, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support a conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to glyphosate administration.

Table 28. Incidence of pancreatic islet cell findings in rats administered glyphosate for 24 months

		Incidence per dietary concentration of glyphosate			
Finding	**Sex**	**0 ppm**	**2 000 ppm**	**8 000 ppm**	**20 000 ppm**
Hyperplasia	M	2/58 (3%)	0/57 (0%)	4/60 (7%)	2/59 (3%)
	F	4/60 (7%)	1/60 (2%)	1/60 (2%)	0/59 (0%)
Adenoma	M	1/58 (2%)	8/57** (14%)	5/60 (8%)	7/59*** (12%)
	F	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)
Carcinoma	M	1/58 (2%)	0/57 (0%)	0/60 (0%)	0/59 (0%)
	F	0/60 (0%)[a]	0/60 (0%)	0/60 (0%)	0/59 (0%)
Adenoma + carcinoma (combined)	M	2/58 (3%)	8/57*** (14%)	5/60 (8%)	7/59 (12%)
	F	5/60 (8%)	1/60 (2%)	4/60 (7%)	0/59 (0%)

ppm: parts per million; **: $P < 0.01$ (Fisher Exact test with Bonferroni inequality); ***: noted to be statistically significant but not analysed in the original report

Results presented as number of rats affected / number of rats examined with the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

There was a statistically significant trend for hepatocellular adenomas in males only, but a significant trend was not seen for adenomas and carcinomas combined ($P > 0.05$) (Table 29). These tumours were not considered to treatment related since 1) their incidences were within the testing facility's historical control range (1–18%); 2) pre-neoplastic lesions (i.e. cell hyperplasia or pre-neoplastic foci) were absent; and 3) there was no evidence of progression to malignancy (adenoma to carcinoma).

An increased incidence of thyroid C-cell adenomas was observed at 8000 and 20 000 ppm in both sexes but this did not reach statistical significance compared to the control animals (Table 29). There was a statistically significant dose trend for C-cell adenomas and adenomas/carcinomas combined in females. The testing laboratory historical control range for C-cell adenomas was 1.8–10.6% for males and 3.3–10% for females; the range for C-cell carcinomas was 0–5.2% for males and 0–2.9% for females. These tumours are not considered relevant to human risk assessment because 1) the increased incidences in males were not statistically significant; 2) there was no evidence of progression from adenoma to carcinoma; 3) and there were no dose-related increases in the incidence or severity of pre-neoplastic lesions (hyperplasia); and 4) they occurred in only one study.

Table 29. Thyroid C-cell tumours in male and female rats administered glyphosate for 24 months

Finding	Sex	Incidence per dietary concentration of glyphosate			
		0 ppm	2 000 ppm	8 000 ppm	20 000 ppm
Adenoma	M	2/54 (4%)	4/55 (7%)	8/58 (14%)	7/58 (12%)
	F	2/57 (4%)*	2/60 (3%)	6/59 (10%)	6/55 (11%)
Carcinoma	M	0/54 (0%)	2/55 (4%)	0/58 (0%)	1/58 (2%)
	F	0/57 (0%)	0/60 (0%)	1/59 (2%)	0/55 (0%)
Adenoma + carcinoma (combined)	M	2/54 (4%)	6/55 (11%)	8/58 (14%)	8/58 (14%)
	F	2/57 (4%)*	2/60 (3%)	7/59 (12%)	6/55 (11%)

F: females; M: males; ppm: parts per million; *: $P < 0.05$ (Cochran–Armitage Trend Test)

Results presented as number of rats affected / number of animals examined, excluding those that died or were terminated prior to study week 55, and the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

The incidence of benign keratoacanthoma was increased in male rats, but as there was no dose–response relationship, it was not considered treatment related (Table 30).

Table 30. Skin keratoacanthoma in male rats administered glyphosate for 24 months

Finding	Incidence per dietary concentration of glyphosate			
	0 ppm	2 000 ppm	8 000 ppm	20 000 ppm
Benign keratoacanthoma (dead and moribund animals)	0/36 (0%)	1/31 (3%)	2/33 (6%)	1/32 (3%)
Benign keratoacanthoma (terminal kill)	0/13 (0%)	2/19 (11%)	2/17 (12%)	2/17 (12%)

ppm: parts per million

Results presented as number of rats with skin keratoacanthoma / number of rats assessed, with the resulting percentage in parentheses.

Source: Strout & Ruecker (1990)

Lymphoma/lymphosarcoma was observed in multiple tissues in male and female rats; however, the incidences in treatment groups were lower than in the controls and no dose relationship was observed.

The NOAEL for toxicity in rats was 8000 ppm (equal to 362 mg/kg bw per day) for decreased body-weight gains in females and cataractous lens changes in males seen at the LOAEL of 20 000 ppm (Strout & Ruecker, 1990).

409

In a combined 2-year chronic toxicity/carcinogenicity study, glyphosate (two batches, purity 98.9 and 98.7%) was fed in the diet to 85 Sprague Dawley rats/sex per dose for 104 weeks in amounts adjusted to deliver 0, 10, 100, 300 and 1000 mg/kg bw per day to both sexes throughout the study. Out of each group of 85 rats, 35 were designated for the toxicity portion of the study while the remainder was designated for the oncogenicity portion of the study. The animals were inspected twice daily for signs of toxicity and mortality. All were clinically examined, including palpitation for tissue masses, prior to the start of the study and weekly thereafter. The animals were weighed and feed consumption measured weekly during weeks 1–13 and once monthly thereafter. Water consumption was inspected throughout the treatment period. An ophthalmoscopic examination was carried out on 20 males and 20 females from each dose group in the oncogenicity study before treatment started and on 20 males and 20 females from the control and high-dose oncogenicity groups at weeks 25 and 51. In addition, all control and high-dose oncogenicity and toxicity study rats were examined at week 102. Blood was collected from the retro-orbital sinus of fasted animals for haematology and clinical chemistry while the animals were under light ether anaesthesia. Samples were obtained from 10 animals/sex per group in the toxicity study at weeks 14, 25, 51, 78 and 102. Urine samples were obtained from 10 animals/sex per group at weeks 14, 26 and 53 in the oncogenicity study and from 10 animals/sex per group at weeks 14, 25, 51, 78 and 102 in the toxicity study. After 52 weeks, 15 males and 15 females from each toxicity study group were terminated and necropsied; all the remaining study animals were terminated and necropsied after 104 weeks. All premature decedents were also necropsied. Selected organs were weighed from all interim kill animals and 10 males and 10 females terminated at the end of the oncogenicity study. All collected tissues from all decedents prior to week 52, those terminated at 52 weeks, and the control and high-dose animals terminated at the end of the study were examined microscopically. Only the salivary glands were examined on the decedents after 52 weeks and the rats from the other dose groups at final termination.

Light-coloured faeces were observed during weeks 16–104 in both sexes at the high dose and in low-mid and high-mid females; however, this sign was not considered toxicologically significant. There were no statistically significant differences in survival rates between each group receiving glyphosate and the control group, in either sex. No treatment-related effect was observed in feed consumption, water consumption and haematology, ophthalmoscopic examinations and gross pathology data. High-dose males had statistically lower mean body weight ($P < 0.01$) by 5–11% from week 2 until week 104; at termination, mean body weight was 10% lower (−14% weight gain). High-dose females had statistically lower body weight ($P \leq 0.05$) by 5–12% from week 20 through week 80 (with several exceptions); at termination, mean body weight was 8% lower (−11% weight gain). Statistically significantly increased alkaline phosphatase activities (+46% to +72%) were observed in high-dose males throughout the study except for week 51 when the mean value was 31% higher than control. Similarly, elevated alkaline phosphatase activities were observed in females at the high dose (+34% to +53%) throughout the study and through most of the study at the high-mid dose (by +20% to +67%, though not always statistically significant). These changes in the alkaline phosphatase activity are considered of little toxicological significance. Urine analysis data showed reduced pH (5.5–6) in males at the high dose throughout the study.

The absolute liver weight was statistically significantly decreased in females at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight, the difference was statistically nonsignificant at all three doses. In males, the absolute liver weight was decreased significantly at 100, 300 and 1000 mg/kg bw per day after 52 weeks, but after correcting for final body weight the difference was also not statistically significant. The parotid salivary-gland weight was increased significantly in males at 100, 300 and 1000 mg/kg bw per day (56–111%) after 52 weeks, but not after 104 weeks; the combined weight of the sublingual and submaxillary salivary glands was significantly increased by 13% (22% after correcting for body weight) at 1000 mg/kg bw per day after 52 weeks. In females, the parotid gland was not affected but the sublingual and submaxillary combined weight was significantly higher by about 15%. The changes in salivary-gland weights were accompanied by increased incidence of mild to severe parotid salivary gland cell alterations and slight to moderate mandibular salivary gland cell alterations in both sexes at week 52 and week 104. The lesions were described as cells and/or acini that appeared larger and stained in a weakly basophilic manner without showing a tendency towards proliferative or degenerative changes

410

over time. In males, the increased incidence and severity of lesions in the parotid gland were significant ($P < 0.01$) at all doses at 52 weeks and at high-mid and high doses at 104 weeks. The increased incidence of lesions in the mandibular gland was significant at high-mid and high doses at 52 weeks and significant ($P < 0.001$) at all doses at 104 weeks. In females, the increased incidence of parotid lesions was significant ($P = 0.001$)at high-mid and high doses at 52 weeks and at all doses at 104 weeks. The increased incidence in the mandibular gland lesions was significant at the high dose at both 52 and 104 weeks. The incidence and/or severity of kidney nephropathy decreased in males at all doses at 52 weeks and at the high dose at 104 weeks. Urothelial hyperplasia was significantly decreased in females from the high-dose group at both the 52-week and 104-week intervals.

Although all groups had neoplastic lesions, none proved to be treatment related when histopathology data from treated groups were compared to that of controls at 104-week termination.

In conclusion, the liver and the salivary glands were identified as the main target organs of glyphosate-related toxicity in the long-term study. At 100 mg/kg bw per day, the changes in salivary glands were only minimal in terms of severity and not considered toxicologically significant. The NOAEL in the 104-week study was 100 mg/kg bw per day in rats based on the more pronounced cellular alteration of salivary glands at 300 mg/kg bw per day and greater. There was no treatment-related increase in tumour incidence at doses up to 1000 mg/kg bw per day (Atkinson et al., 1993b).

In a dietary toxicity study in rats, groups of 24 male and 24 female Alpk:AP$_f$SD (Wistar-derived) rats were fed diets containing glyphosate (purity 95.6%) at concentrations of 0, 2000, 8000 or 20 000 ppm (equal to 0, 141, 560 and 1409 mg/kg bw per day for males and 0, 167, 671 and 1664 mg/kg bw per day for females) for 1 year. Analysis of diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study. The animals were monitored daily for mortality and clinical signs. Body weights and feed consumption were measured at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination, and the rats were terminated and necropsied. Blood and urine samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

None of the pre-terminal deaths during the study could be attributed to the administration of glyphosate. Apart from a small increase in the number of high-dose male and female animals that showed wet or dry urinary staining, no treatment-related clinical changes were seen. In addition, there were no treatment-related ophthalmological findings. Body weights of high-dose animals were lower than concurrent controls throughout the study; body weights of animals at 8000 ppm were slightly reduced (but not significantly in males and significantly only from week 46 in females). There was no effect on body weight in animals on 2000 ppm glyphosate. The changes in body weights in males and females were not considered biologically significant since the magnitude of change was small (less than 10%).

Feed consumption was lower and feed utilization was slightly less efficient at 20 000 ppm, the reductions being most marked at the start of the study. There was a trend for reduced feed intake for females at 8000 ppm, which correlates with the reduction in body-weight gain at this dose in the latter stages of the study.

Some statistically significant differences in haematological parameters were seen between treated and control animals, but the differences were small and inconsistent across the various time points, and were considered unrelated to the administration of glyphosate. Deviations in some clinical chemistry parameters, such as reductions in plasma concentrations of cholesterol and triglycerides or a dose-related increase in plasma alkaline phosphatase activity throughout the study as well as occasional increases in the activities of plasma aspartate aminotransferase, alanine transaminase and creatine kinase, were mostly confined to high- and intermediate-dose groups. In the absence of any histopathological findings these marginal changes are not considered toxicologically significant.

There was no evidence of any effect of glyphosate on urine parameters. At necropsy, there were no treatment-related gross pathological findings or consistent organ-weight changes. An increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland

were seen in both sexes at 20 000 ppm. At 8000 ppm, examples of focal parotid basophilia were of minimal severity and the incidence was slightly above that in the control animals. No other microscopic findings could be ascribed to administration of glyphosate.

Similar numbers and types of neoplasms were diagnosed in the control group and in the 20 000 ppm group, but the study was too short to be able to reach any conclusions about carcinogenicity.

The NOAEL for the increased incidence of basophilia of parotid acinar cells in the 1-year toxicity study in rats was 8000 ppm (equal to 560 mg/kg bw per day) based on the increased incidence of basophilia of parotid acinar cells at 20 000 ppm (Milburn, 1996).

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 96.8 and 90.0%, two batches) was fed in the diet to 50 Wistar rats per sex per dose for up to 2 years at concentrations of 0, 100, 1000 or 10 000 ppm (equal to 0, 6.3, 59.4 and 595.2 mg/kg bw per day for males and 0, 8.6, 88.5 and 886 mg/kg bw per day for females). In addition, one vehicle control (acetone) group with 10 rats per sex and one high-dose group with 20 rats per sex were included for interim termination at the twelfth month to study non-neoplastic histopathological changes. Veterinary examinations took place before and after grouping and at the end of each month of experimental schedule. Individual body weights were recorded before dosing, at weekly intervals until the end of week 13 and every 4 weeks thereafter until termination. Feed consumption was recorded once weekly for each cage group from week 1 to week 13 and subsequently over 1 week in every 4 weeks until termination. Individual blood samples were collected from 20 rats/sex per group at 3, 6, 12, 18 and 24 months. At scheduled intervals of 6, 12, 18 and 24 months, blood collected from 10 rats/sex per group underwent clinical chemistry analysis. Individual urine samples were collected from 10 rats/sex per group at 3, 6, 12, 18 and 24 months. Histopathological examination was carried out on all tissues collected at interim termination of control and high-dose groups; on all pre-terminally dead and moribund terminated rats in the low- and mid-dose groups; and on all lesions of the terminated rats from the low- and mid-dose groups. Selected organs from 10 rats/sex per dose were weighed. The stability of glyphosate was determined at 2000 and 20 000 ppm which demonstrated that prepared diets were fairly stable for 30 days at room temperature with a degradation of less than 7% of the pure compound. The analysis of diets indicated that the achieved concentrations were within acceptable range. There were no treatment-related effects on mortality, clinical observations, body weights, body-weight gains, feed consumption, urine analysis and haematology. The following significant ($P < 0.05$) dose-related changes in blood chemistry parameters were seen at the high dose: decrease in gamma-glutamyltransferase levels at 12 months in male rats; a decrease in albumin levels at 6 months in female rats; and increases in alkaline phosphatase levels at 6, 12 and 18 months in female rats. The increase in alkaline phosphatase in high-dose females were 235, 231, 194, and 249 (U/L) at 6, 12, 18 and 24 months, respectively, while the corresponding control values were 133, 141, 101, 254 for females at 6, 12, 18 and 24 months, respectively.

Neither treatment-related macroscopic findings nor changes in organ weights or relative organ weights were observed during the study period. None of the significant microscopic changes or increased and decreased incidences (in liver, spleen, lymph nodes, adrenals, thymus, gonads, uterus, mammary gland) showed dose relationships, indicating that they were incidental and not related to the treatment with the glyphosate. At terminal kill, the incidence of cataracts in males at 0, 100, 1000 and 10 000 ppm was 3/20, 3/20, 1/18 and 6/29, respectively, while in females it was 1/24, 1/26, 5/33 and 4/21, respectively. The historical data on neoplasm incidence for the test species indicates that the incidences of the various tumours observed are within the normal range. The types of tumours seen were also comparable to the historical records. No statistically significant intergroup difference between the control and low-, mid- and high-dose treatment groups was recorded in terms of the number of rats with neoplasms, number of malignant neoplasms and incidence of metastasis either sex-wise or for combined sex.

412

The NOAEL in this combined chronic toxicity/carcinogenicity study in rats was 10 000 ppm, the highest dose tested, equal to 595.2 mg/kg bw per day. There was no evidence of carcinogenicity of glyphosate at doses up to 10 000 ppm in rats at in this study (Suresh, 1996).

In a combined chronic toxicity and carcinogenicity study, groups of 50 Sprague Dawley rats per sex were fed daily dietary doses of 0, 3000, 15 000 and 25 000 ppm (equal to 0, 180, 920 and 1920 mg/kg bw per day for males and 0, 240, 1130 and 2540 mg/kg bw per day for females) glyphosate technical for 2 years. In addition, 20 rats/sex per dose were included for interim termination in week 52 as part of the chronic toxicity study to study non-neoplastic histopathological changes; the dose levels were the same except the highest dose was 30 000 ppm. Test diets were prepared weekly by mixing appropriate amounts of the test material with the basal diet. The stability and homogeneity of the test material in feed was determined in an in-house stability study at all dose levels before the start of dosing. Analyses for achieved concentrations were performed monthly during the study period.

No treatment-related clinical signs or deaths were observed in the 52-week chronic toxicity study. In the 104-week carcinogenicity study, male animals of the high-dose group exhibited slight but statistically insignificant higher mortalities. No significant toxic signs were observed in treated or control groups. Significantly reduced body-weight gain that lasted throughout the study was observed in high-dose males. In all other groups, body-weight gain at termination was comparable to the control. No treatment-related effects on feed consumption for either sex or any group were noted during the study. The results show a higher intake for females compared to males for each dose level. The mean intake in the chronic toxicity study was 0.18, 0.92 and 1.92 g/kg bw per day (males) and 0.24, 1.13 and 2.54 g/kg bw per day (females) for 3000, 15 000 and 30 000 ppm, respectively. The mean intake in the carcinogenicity study was 0.15, 0.78 and 1.29 g/kg bw per day (males) and 0.21, 1.06 and 1.74 g/kg bw per day (females) for 3000, 15 000 and 25 000 ppm, respectively.

Ophthalmological examinations revealed no abnormalities. Haematological examination showed no treatment-attributable abnormalities. A significant increase in the alkaline phosphatase level was only seen at 25 000 ppm in the carcinogenicity study at study termination. Other significant changes observed in haematological and biochemical parameters were within the range of the historical control data, indicating that they were of no biological significance. Urine analysis did not reveal any treatment-attributable abnormalities. No treatment-related macroscopic findings were observed during the study period.

Significant and dose-dependent effects were found in high-dose males and females in the chronic toxicity study. In males, weights of kidneys, brain and testes were increased; in females, in addition to increased weights of kidneys and brain, liver weight was also increased.

Histopathological changes were found at all dose levels including the control, indicating that these are no treatment-related effects. There were no treatment-related neoplasms observed.

Based on mild effects on body-weight gain and the increased organ weights without histopathological changes, the NOAEL in rats after chronic exposure to glyphosate technical for 24 months was 15 000 ppm (920 mg/kg bw per day) (Bhide, 1997).

In a 2-year combined chronic toxicity and carcinogenicity study, groups of 50 Sprague Dawley rats/sex per group were fed daily dietary doses of HR-001 at concentrations of 0, 3000, 10 000 or 30 000 ppm (equal to 0, 104, 354 and 1127 mg/kg bw per day for males and 0, 115, 393 and 1247 mg/kg bw per day for females) for 24 months. In addition, 30 rats per sex per group were included for interim termination at 26, 52 and 78 weeks.

At 3000 ppm, males exhibited significant increases in incidence of decreased spontaneous motor activity, bradypnea and soiled fur (predominantly in external genital area and foreleg) and a significant decrease in incidence of tactile hair loss. Females at 3000 ppm showed significant increases in incidence of ptosis and tactile hair loss. At 10 000 ppm, the incidence of tactile hair loss

was significantly decreased in males and significantly increased in females compared to their respective controls.

At 30 000 ppm, neither sex showed an increase in mortality, although mortality in males was lower than the control during the last half of the treatment period, with statistical significance most weeks. In all other groups, mortality was comparable to the control. Males had significant increases in incidence of bradypnea, palpable masses and soiled fur (at the external genital or perianal region) compared to controls. Palpable masses in the tail were present in 27 males, a high incidence compared to 11 for the controls; the incidences of masses in other locations were comparable to the controls. Males at 30 000 ppm also showed significant decreases in incidence of tactile hair loss, incidence of wounds and hair loss. In females, a significant increase in incidence of wet fur, mainly in the external genital area, was observed. In addition, loose stools were observed in all cages from week 24 in males and week 23 in females until the end of the treatment.

There was an increase in benign keratoacanthoma in males at 24 months that was statistically significant in trend wise comparison but not in pair wise comparison (Table 31). However, skin keratoacanthoma is one of the most common spontaneous benign neoplasms in male Sprague Dawley rats (Chandra, Riley & Johnson, 1992). Adenomas of the kidney were observed in four males in the 30 000 ppm grouped compared to zero in the controls. The background incidence of this tumour in this strain of rat is reported to be 0.7% (0–2.9%), and the incidence of the tumour in the 30 000 ppm group was only slightly higher than this background incidence. Because there was no statistically significant difference in incidence between the control and the 30 000 ppm group, the slightly higher incidence was not considered due to the treatment with glyphosate.

Table 31. Skin keratoacanthoma in male rats administered HR-001 for 24 months

	Incidence per dietary concentration of HR-001			
Finding	**0 ppm**	**3 000 ppm**	**10 000 ppm**	**30 000 ppm**
Benign keratoacanthoma (dead and moribund animals)	2/32 (6%)	1/30 (3%)	0/32 (0%)	1/21 (5%)
Benign keratoacanthoma (terminal kill)	1/18 (6%)	2/20 (10%)	0/18 (0%)	6/29 (21%)

ppm: parts per million

Results presented as number of male rats with skin keratoacanthoma / number assessed, with resulting percentage in parentheses.

Source: Enomoto (1997)

The NOAEL for chronic toxicity was 3000 ppm (104 mg/kg bw per day) and the LOAEL 10 000 ppm (354 mg/kg bw per day) based on an increase in ptosis and of tactile hair loss in female rats in 24-month study. There was an increased incidence of multiple clinical signs at 30 000 ppm (Enomoto, 1997).

In a combined chronic toxicity and carcinogenicity study, groups of Fischer F344/DuCrlCrlj rats (50/sex per dose) were fed diets containing glyphosate (purity 97.5%) at concentrations of 0, 500, 4000 or 32 000 ppm (equal to 0, 25, 201 and 1750 mg/kg bw per day for males and 0, 29.7, 239 and 2000 mg/kg bw per day for females) for 104 weeks. An interim termination was conducted on 14 rats per sex per dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in diet determined. Clinical observations (including ophthalmoscopy), body weights, feed consumption, haematology and clinical biochemistry (blood and urine) were measured throughout the study. A functional observational battery, including motor activity, was conducted in week 52 in animals allocated to the chronic toxicity assessment of the study. At the end of the scheduled period the animals were terminated and necropsied. Blood samples were taken for

clinical pathology, selected organs weighed and specified tissues prepared for subsequent histopathological examination.

Prepared diets were stable at room temperature for 4 months and the test material was homogeneously distributed in the diet. Analysis of the prepared diet indicated that the measured concentrations ranged from 80–110% of the nominal concentrations. All males and females at 32 000 ppm had diarrhoea or soft stools from immediately after the start of administration and throughout the administration period. Mortality was not affected. Statistically significantly reduced body weights were observed throughout the study in high-dose males (beginning week 1) and females (beginning week 2). Feed consumption in all dosed group decreased or increased (no statistical significance) at various intervals. The only treatment-related effects observed in urine analysis were increased urinary proteins in three high-dose females at week 104. These changes were thought to be related to the histological changes in the kidney. There were no remarkable changes in females at any other dose or other examination time or in males at any dose. Males and females at 32 000 ppm showed statistically significant decreases or tendencies towards decreases in erythrocyte count, haematocrit and haemoglobin concentration in weeks 26, 52 and 78, and males in this group also showed significant increases in platelet count and leukocyte count in week 52 and a significant increase in platelet count in week 78. At 4000 ppm, females showed a significant decrease in erythrocyte count in week 26 (94% of the control value) and males showed significant decreases in erythrocyte count (96% of the control value) and haematocrit (95% of the control value) in week 52. In males and females at 500 ppm, there were no significant differences compared to the controls at 0 ppm in any examination parameter. The historical control values for haematological parameters from the performing laboratory were not available, however, and the historical control data for Fisher Inbred Strain F344/DuCrlCrlj were used to compare with study results. Throughout the study, except at week 104, the control group had higher erythrocyte counts and haematocrit values than the range reported in the literature for this strain of rats. This suggests that erythrocyte and haematology values for the control groups of the TAC study were unusually high, and that statistically significant decreases in test groups may not be toxicologically significant or relevant. Males and females at 32 000 ppm showed a tendency towards a decrease in albumin at each examination time, and the values were statistically significant in males and females in week 26 and in males in week 78 compared to controls. In addition, males in this group showed significant increases in gamma-glutamyltransferase, alkaline phosphatase and total bilirubin in week 52. Otherwise the following changes were not observed continuously or at 32 000 ppm and were therefore considered unrelated to administration of the test material: significant decreases in creatinine, alanine transaminase [serum glutamic pyruvic transaminase] and total bilirubin in males or females in week 26 at 32 000 ppm and significant increases in creatinine, total protein and albumin in females at 500 ppm. Ophthalmoscopic examination indicated treatment-related opacity in one high-dose female at week 104 but was considered incidental. At 32 000 ppm, a statistically significant increase in relative kidney weights was observed in males after the scheduled termination in week 79 and in males and females at the scheduled termination in weeks 105–106. Otherwise, the following changes were recorded, but were thought to be due to suppressed body-weight gain as there were no corresponding abnormalities in histopathological examination: significant increases in the relative weights of the brain and liver in males at the week 79 and week 105–106 scheduled terminations and females in the week 105–106 scheduled termination; a significant decrease in the absolute weight of the adrenal in high-dose males in the week 105–106 scheduled termination; and a significant decrease in the absolute weight of the brain in mid-high (4000 ppm) males in the week 79 scheduled termination. High-dose males and females showed an increase in luminal dilatation of the large intestine at necropsy at the week 79 termination, but there were no histological changes. Thymic involution increased in all females at 32 000 and 500 ppm. However, these effects were thought to be incidental since they are age-related changes.

Histopathological examination showed an increase in glomerulosclerosis in females at 4000 ppm and 32 000 ppm during the scheduled necropsy at week 105–106 and increases in eosinophilic granule/hyaline droplets in the tubular epithelium in the kidney in females at the week 79 necropsy and in males and females at the week 105–106 scheduled necropsy. Monsanto and TAC co-sponsored the PWG to re-evaluate the microscopic kidney findings, specifically glomerulosclerosis, chronic

nephropathy and hyaline droplet renal tubule degeneration in female rats. The PWG concluded (Hardisty, 2013) that the kidneys of male and female rats did not confirm the study pathologist's reported conclusions that the incidence of glomerulosclerosis and the presence of eosinophilic granules/hyaline droplets of renal tubule epithelium were treatment related. The PWG found no histological evidence of renal toxicity in the sections of kidneys examined. The only frequently observed finding in the kidneys of male and female rats was chronic progressive nephropathy which, however, was similar in incidence and severity in control and treated groups. No treatment-related tumours were observed.

In conclusion, the NOAEL for chronic toxicity of glyphosate in rats was 4000 ppm (equal to 201 mg/kg bw per day) based on the decrease in body weights, transient haematological effects, diarrhoea, urine parameters, clinical chemistry effects, increased kidney weight relative weight seen at 32 000 ppm, the highest dose tested, in this 104-week study. Glyphosate was not carcinogenic in rats at doses up to 32 000 ppm (Takahashi, 1999b).

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 97.6%) was fed to 64 Alpk:AP$_f$SD Wistar-derived rats per sex per dose in the diet for up to 2 years at concentrations of 0, 2000, 6000 or 20 000 ppm (equal to 0, 121, 361 and 1214 mg/kg bw per day for males and 0, 145, 437 and 1498 mg/kg bw per day for females). An interim termination was conducted on 12 rats per sex per dose after one year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in the diet determined. Clinical observations (including ophthalmoscopy), body weights, feed consumption, haematology and clinical biochemistry (blood and urine) were conducted throughout the study. A functional observational battery, including motor activity, was conducted in week 52 in animals allocated to the chronic toxicity assessment part of the study. At the end of the scheduled study period, the animals were terminated and necropsied. Cardiac blood samples were taken for clinical pathology, selected organs weighed and specified tissues taken for subsequent histopathological examination.

The mean achieved concentrations of glyphosate in each dietary preparation were within 10% of the nominal concentration, and the overall mean concentrations were within 1% of nominal. The diets were homogenously distributed and prepared diets were stable at room temperature for 45 days. Survival in control, low- and mid-dose males approached 25% by week 104 of the study (criteria for termination of the study) although survival in the high-dose group was significantly better. Survival in the females was similar across all groups and better than in the lower-dose males. Treatment-related increase in the incidence of red-brown staining of tray papers (particularly in males) and isolated observations of red/brown coloured urine were noted in three males and one female at 20 000 ppm. The body weights of the high-dose rats were statistically significantly lower than controls throughout the study; however, these differences were not considered toxicologically relevant since maximum decreased in body weights were approximately 5% and 8% for males and females, respectively. Feed consumption and feed utilization were statistically significantly lower in high-dose males and females. Ophthalmoscopic examination did not reveal any treatment-related effects, and no treatment-related observations were noted in the functional observational battery, grip strength measurements, motor activity, landing foot splay measurements and time to tail flick. Haematological parameters were not affected by the treatment. Statistically significant increases in alkaline phosphatase activity occurred at all doses in both sexes up to week 79. There was evidence at one or more time points of increases in plasma alanine transaminase and aspartate aminotransferase activities and total bilirubin, but statistical significance was reached only at 6000 and 20 000 ppm. In the absence of any histopathological findings these marginal changes are not considered toxicologically significant. Plasma triglycerides and cholesterol were consistently decreased for all or part of the study in males at 20 000 ppm. Plasma creatinine values were lower in all treated female groups at week 27 and in females at 6000 and 20 000 ppm at week 14, but in the absence of any effects later in the study, this is considered not toxicologically significant. Urinary pH was lower than that of controls in high-dose males throughout the study. An increase in the incidence and severity of blood/red blood cells was seen in males and, to a lesser extent, in females at 20 000 ppm. There were no consistent, dose-related effects on organ weights indicative of a toxicologically significant effect of glyphosate.

Macroscopic findings consisting of a minor increase in incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes were seen in males at 6000 and 20 000 ppm. A minor increase in the incidence but not the severity of proliferative cholangitis in the liver was observed at interim and terminal kills in high-dose males. Moreover, an increased incidence of hepatitis and periodontal inflammation was observed in high-dose males. There were a number of changes in the kidneys of high-dose males and females, notably renal papillary necrosis, with or without papillary mineralization, and transitional cell hyperplasia; the incidence was greater in males than females. These findings are considered treatment related but are consistent with ingesting high doses of an acidic material, which may also have caused the microscopically observed prostatitis and periodontal inflammation. The decrease in the incidence of tubular degeneration of the testis in high-dose males is considered of no consequence (Table 32). The incidence of prostatitis was higher than the control groups in all treated males but it was within historical background levels in all treated groups; however, as the control value in this study was low, the relationship to treatment at the high-dose level cannot be entirely dismissed.

Table 32. Selected microscopic findings in rats administered glyphosate for 2 years

	No. per dietary concentration of glyphosate							
	Males				Females			
Organ / Finding	**0 ppm**	**2 000 ppm**	**6 000 ppm**	**20 000 ppm**	**0 ppm**	**2 000 ppm**	**6 000 ppm**	**20 000 ppm**
Liver: Proliferative cholangitis	56	57	55	64	55	58	59	61
Liver: Hepatitis	8	6	9	13	6	7	4	6
Kidney: Papillary necrosis	0	1	0	14	0	1	2	5
Kidney: Transitional cell hyperplasia	2	3	0	5	3	1	0	1
Prostate: Prostatitis	13	22	23	37	–	–	–	–
Testis: Unilateral tubular degeneration	18	13	18	5				
Periodontal inflammation	25	27	23	42	18	24	32	28

no. number; ppm: parts per million

Results presented as number of rats with the finding. $N = 64$ for male and for female rats.

Source: Brammer (2001)

In contrast to a previously described 1-year feeding study in rats (Milburn, 1996), microscopic changes were seen in the liver and kidneys of high-dose rats but not the salivary glands, even though the study was conducted on the same strain of the rats and in the same laboratory.

The incidence of hepatocellular adenomas in male rats at the high dose increased compared to the controls (0/52 at 0 ppm, 2/52 [4%] at 2000 ppm, 0/52 [0%] at 6000 ppm and 5/52 [10%] at 20 000). However, this increase was considered incidental rather than treatment related, for the following reasons: 1) the absence of a dose–response relationship; 2) the lack of progression to malignancy; 3) no evidence of pre-neoplastic lesions; 4) the incidences were within the range (0–11.5%) of historical controls for this strain (Wistar) of rats in 26 studies conducted between 1984 and 2003 at the testing laboratory; and 5) the 0% incidence in the concurrent controls is lower than the average background incidence for liver adenomas in male Wistar rats, which distorts the comparison.

In conclusion, the NOAEL for chronic toxicity of glyphosate in rats was 6000 ppm (equal to 361 mg/kg bw per day) based on kidney, prostate and liver toxicity seen at 20 000 ppm (equal to 1214 mg/kg bw per day) in this 2-year study. There was no evidence of carcinogenicity in rats at glyphosate doses up to 20 000 ppm (Brammer, 2001).

417

In a combined chronic toxicity/carcinogenicity study, glyphosate (purity 95.7%) was fed to Han Crl:WI (GLx/BRL/HAN) IGS BR Wistar rats (51/sex per dose) in the diet for up to 104 weeks at concentrations of 0, 1500, 5000 or 15 000 ppm (equal to mean achieved doses of 0, 95.0, 316.9 and 1229.7 mg/kg bw per day). To ensure that a dose of 1000 mg/kg bw per day overall was received, the highest dose was progressively increased to 24 000 ppm. In addition, three satellite groups with 15 rats per sex each were included for interim termination at the twelfth month to study non-neoplastic histopathological changes. A satellite control group with 12 rats per sex served as veterinary control; these animals were to be used for investigations should any health problems have developed with the study animals. As no such problems occurred, observations of these animals have not been included in the report.

The prepared diets were stable for at least 6 weeks and their achieved dietary concentrations were within acceptable ranges.

Clinical signs, functional observations, body-weight changes and feed and water consumption were monitored throughout the study. Clinical chemistry and haematological examinations were performed on 10 animals per sex from the satellite and main groups at 3, 6 and 12 months. More haematological and clinical chemistry investigations were performed on 20 animals per sex from the main groups at 18 and 24 months. Urine analysis of 10 animals per sex from satellite groups at 3, 6 and 12 months and from main groups at 18 and 24 months was conducted. All survivors at study termination (main groups: 104 weeks; satellite groups: 52 weeks) were necropsied as were all pre-terminal decedents or those terminated in extremis. Selected organs of 10 animals/sex per group terminated at the end of the study and all the animals from satellite groups were weighed. Histopathological examination was initially carried out on all tissues collected from control and high-dose groups; all pre-terminally dead and moribund euthanized rats and on all lesions and palpable masses of the terminated rats from the low- and mid-dose groups. Since there were no indications of treatment-related bone marrow changes, examination was subsequently extended to the remaining treatment groups.

No significant treatment-related effects were observed on mortality, clinical signs, behavioural assessments, functional performance tests (motor activity, grip strength values), sensory reactivity, body weights, body-weight gains, feed consumption, water consumption, palpable masses, ophthalmoscopic examinations, haematology, clinical chemistry, urine analysis, organ weights and macroscopic findings.

Adipose infiltration of bone marrow was seen in the majority of animals examined, with both sexes being more or less equally affected in terms of incidence and severity. However, generally greater effects were seen in male rats at 15 000 ppm and this attained statistical significance for terminal kill animals, indicating the possibility of myeloid hypoplasia as a consequence of treatment. However, given the normal variability of this condition and the effect of other pathological conditions upon marrow cellularity in ageing rats, the effect – although not altogether convincing – cannot be dismissed as a similar effect was not seen in male rats in the remaining treatment groups. A higher incidence of higher grades of severity of adipose infiltration was seen in premature decedents of both sexes at 5000 ppm and females only at 1500 ppm. However, the variable duration of exposure and significant background pathology for pre-terminal decedents further negates this as an effect of treatment upon marrow cellularity for female rats.

At the highest dose, differences in the site of mineral deposition in the kidneys were significant compared with controls. Pelvic mineralization was commonly seen in both sexes and was more prevalent in female rats; however, corticomedullary mineralization was seen in female rats only. Nephrocalcinosis in rats is generally considered to be related to diet and hormonal status. There was a lower incidence of pelvic/papillary deposition and an increase in the corticomedullary deposition. At the same time the incidence of renal pelvic hyperplasia was reduced in in both sexes as a consequence of the decreased mineral deposition. The effects on pelvic and corticomedullary mineralization as well as hyperplasia of the pelvic/papillary epithelium were confined to high-dose animals and there was no indication of a similar effect at any other treatment level for either sex.

Treatment did not affect the development of neoplasia in any organ or tissue or the overall frequency of benign or malignant tumours.

In conclusion, the NOAEL in rats after chronic exposure to glyphosate technical for 24 months was 15 000 ppm (equal to mean achieved dose level of 1229.7 mg/kg bw per day), the highest dose tested. Glyphosate was not carcinogenic in rats at doses up to and including 15 000 ppm, the highest dose tested (Wood et al., 2009b).

In a published drinking water study, ammonium salt of glyphosate (13.85% solution) was administered to groups of 85 male and 85 female Wistar–RIZ rats in drinking water at concentrations of 0, 300, 900 or 2700 mg/L for 2 years. Examination of peripheral blood parameters and bone marrow smears did not reveal any harmful effects. In addition, there was no treatment-related effects on the blood or urine biochemical parameters evaluated. The study authors concluded that glyphosate has no effect on neoplastic pathogenesis (Chruscielska et al., 2000a). The study report lacks detailed information on the formulated product or detailed description of the methodology, histopathological examination and tumour description.

In a published study, the health effects of a Roundup-tolerant NK603 genetically modified maize (from 11% in the diet), cultivated with or without Roundup application and Roundup alone (from 0.1 parts per billion [ppb] of the full pesticide containing glyphosate and adjuvants) in drinking water, were evaluated for 2 years in groups of 10 male and 10 female rats/dose. This study was used to evaluate the long-term toxicity and was not a carcinogenicity evaluation. The test material is a formulated product and the study report lacked details of the results (Séralini et al., 2014).

2.4 Genotoxicity

Glyphosate and its formulation products have been extensively tested for genotoxic effects using a variety of end-points in a wide range of organisms. These tests have ranged from standard, validated tests in bacteria and mammalian model organisms to less common and non-validated tests in phylogenetically distant species such as plants, earthworms, clams, frogs, tropical fish and caimans. In these studies, the test materials were administered through a variety of routes including parenteral routes used for specialized studies but considered largely irrelevant for assessing risks resulting from low-level dietary exposures. The reviewed studies for glyphosate are briefly summarized in the text and tables below (genotoxicity studies on AMPA, *N*-acetyl-glyphosate, *N*-acetyl-AMPA and other formulation ingredients are in Section 2.7, 2.8 and 2.9). Summary tables of studies conducted in non-traditional or phylogenetically distant organisms are shown in Appendix 1. In addition, a number of studies were conducted of humans exposed occupationally or environmentally to glyphosate and/or its formulation products. Many of these involved co-exposures to many different pesticides and were considered uninformative; however, the few studies that considered glyphosate the major agent are summarized and briefly discussed below.

A much smaller number of studies have been conducted on the glyphosate metabolite, AMPA, as well as the plant metabolites, *N*-acetyl-glyphosate and *N*-acetyl-AMPA. The results are shown in Tables 33, 34 and 35. The in vivo studies (Table 35) investigated the ability of these metabolites to induce micronuclei in the bone marrow erythrocytes of mice and have largely been negative although a modest positive response was reported by Manas (2009b) when AMPA was administered in male mice by intraperitoneal injection. Studies by other investigators using the more relevant oral route of administration did not show an increase in micronuclei in either male or female mice.

In the in vitro studies, increases in mutation in bacteria were not seen for AMPA or the acetylated metabolites. Both positive and negative results were reported in studies of chromosome aberrations and DNA damage for AMPA. AMPA was negative in two studies of unscheduled DNA

synthesis in isolated rat hepatocytes. Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

(a) In vitro studies

Bacteria

Glyphosate or Roundup was used in approximately 40 studies of mutagenicity in bacteria. Most were conducted with and without metabolic activation (using S9, 9000 × *g* supernatant fraction from induced male rat liver homogenate). The actual number of tests performed was well over 150 as multiple tester strains with and without S9 were used in most studies. Glyphosate or Roundup was found to be negative for genotoxic effects in almost all of these; weak positive results were reported in only one or two studies. Glyphosate was also reported to be negative in three assays measuring DNA repair (rec) in *Bacillus subtilis* and positive in one SOS-chromotest assay in *Escherichia coli*. Several studies reported that glyphosate could enhance DNA strand breaks or interfere with DNA strand break repair in cyanobacteria following exposure to ultraviolet-B radiation.

In the case of AMPA or the acetylated metabolites, no increases in mutation in bacteria were seen in the in vitro studies (Table 33).

Table 33. Summary of in vitro genotoxicity studies with glyphosate, glyphosate formulations, AMPA or their metabolites in bacteria

End-point	Test object	Concentration	Purity	GLP (Yes/No)	Results –S9	Results +S9	Reference
Point mutations	*Salmonella typhimurium* TA98, 100, 1535, 1537	0.1–1 000 µg/plate	Glyphosate (98.4%)	No	Negative	Negative	Kier (1978)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538	0.005–50 µL/plate	Glyphosate trimesium SC-0224 (19.2%)	Yes	Negative	Negative	Majeska (1982)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	10– 5 000 µg/plate	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	1.6–5 000 µg/plate	Glyphosate trimesium ICIA 0224	Yes	Negative	Negative	Callander (1988a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	313–5 000 µg/plate	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Negative	Yanagimoto (1991)
Point mutations	*S. typhimurium* TA98, 100, 1535 and 1537	160–5000 µg/plate	Glyphosate (98.6%)	Yes	Negative	Negative	Jensen (1991a)
Point mutations	*S. typhimurium* TA97, 98, 100, 1535	33–10 000 µg/plate	Glyphosate (98.6%)	No	Negative	Negative	Chan & Mahler (1992)
Point mutations	*S. typhimurium* strains TA98, 100, 1535, 1537	50–5 000 µg/plate	Rodeo (40% glyphosate)	Yes	Negative	Negative	Kier et al. (1992)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results –S9	Results +S9	Reference
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2, WP2 uvrA	100–5 000 µg/plate	Glyphosate trimesium TMSC (95%)	Yes	Negative	Negative	Callander (1993)
Point mutations	*S. typhimurium* TA98, TA100	180–1 440 µg/plate	Roundup	No	Weak positive / equivocal	Weak positive / equivocal	Rank et al. (1993)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537	156–5 000 µg/plate	HR-001 (95.7%)	Yes	Negative	Negative	Akanuma (1995a)
Point mutations	*S. typhimurium* strains TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	50–5 000 µg/plate	Glyphosate (95.3%)	Yes	Negative	Negative	Thompson (1996)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2,WP2 uvrA	100–5 000 µg/plate	Glyphosate (95.6%)	Yes	Negative	Negative	Callander (1996)
Point mutations	*S. typhimurium* TA97a, 98, 100, 1535	1–5 000 µg/plate	Glifos (360 g/L glyphosate)	No	Negative	Negative	Vargas (1996)
Point mutations	*S. typhimurium* TA97a, 98, 100, 102	0.025–0.3 µg/plate	Glyphosate formulation Perzocyd 10, soluble liquid concentrate	No	Negative	Negative	Chruscielska et al. (2000b)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	10–5000 µg/plate	Glyphosate technical (97%)	Yes	Negative	Negative	Schreib (2012)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	648–5000 µg/plate	Glyphosate technical Helm (98%)	Yes	Negative	Negative	Riberri do Val (2007)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (95.1%)	Yes	Negative	Negative	Sokolowski (2007a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (97.7%)	Yes	Negative	Negative	Sokolowski (2007b)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (95%)	Yes	Negative	Negative	Sokolowski (2007c)
Point mutations	*S. typhimurium* TA97a, 98, 100, 102, 1535	1–1000 µg/plate	Glyphosate TC (98%)	Yes	Negative	Negative	Miyaji (2008)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–3160 µg/plate	Glyphosate TC (97.5%)	Yes	Negative	Negative	Flügge (2009a)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results		Reference
					−S9	+S9	
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2, WP2 uvrA	3–5000 µg/plate	Glyphosate (96.3%)	Yes	Negative	Negative	Sokolowski (2009)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–5000 µg/plate	Glyphosate (> 96%)	Yes	Negative	Negative	Donath (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–3160 µg/plate	Glyphosate TC (95.2%)	Yes	Negative	Negative	Flügge (2010)
Point mutations	*S. typhimurium* A98, 100, 1535, 1537; *E. coli* WP2 uvrA	31.6–5000 µg/plate	Glyphosate (96%)	Yes	Negative	Negative	Schreib (2010)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	3–5000 µg/plate	Glyphosate (> 95%) spiked with glyphosine (0.63%)	Yes	Negative	Negative	Sokolowski (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	31.6–5000 µg/plate	Glyphosate (> 95.8%)	Yes	Negative	Negative	Wallner (2010)
Point mutations	*S. typhimurium* TA98, 100, 102, 1535, 1537	10–2000 µg/plate	Glyphosate (> 95.4%)	Yes	Negative	Negative	Donath (2011a)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate (98.8%)	Yes	Negative	Negative	Donath (2011b)
Point mutations	*S. typhimurium* TA98, 100, 1535 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate (97.8%)	Yes	Negative	Negative	Donath (2011c)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	1.5–5000 µg/plate	Glyphosate (85.8%)	Yes	Negative	Negative	Thompson (2014)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	10–5000 µg/plate	Glyphosate technical (94.1%)	Yes	Negative	Negative	Schreib (2015)
DNA damage	*B. subtilis* Rec assay H17 and M45	20–2 000 µg/disk	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
DNA damage	*B. subtilis* Rec assay H17 and M45	15–240 µg/disc	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Negative	Yanagimoto (1992b)
DNA damage	*B. subtilis* Rec assay H17 and M45	7.5–240 µg/disk	Glyphosate (95.7%)	Yes	Negative	Negative	Akanuma (1995b)

422

End-point	Test object	Concentration	Purity	GLP (Yes/No)	Results –S9	Results +S9	Reference
DNA damage	*E. coli* SOS chromotest	0.1–0.25 µg	Roundup	No	Positive	N/A	Raipulis et al. (2009)
Enhanced UV-induced DNA strand breaks	Cyanobacteria (*Scytonema javanicum*)	10 µmol/L	Glyphosate	No	Positive	Negative	Wang et al. (2012)
Delayed UV–B-induced DNA strand break repair	Cyanobacteria (*Anabaena* sp.)	10 µmol/L	Glyphosate	No	Positive	N/A	Chen et al. (2012)
Delayed UV-B-induced DNA strand break repair	Cyanobacteria (*Microcystis viridis*)	10 µmol/L	Glyphosate	No	Positive	N/A	Chen et al. (2012)
DNA damage	Acellular prophage superhelical PM2 DNA	75 mmol/L	Glyphosate (98.4%)	No	Negative	N/A	Lucken et al. (2004)
AMPA							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	200–5 000 µg/plate	AMPA (99.3%)	Yes	Negative	Negative	Akanuma (1996)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537, 1538; *E. coli* WP2 uvrA	1.6–5 000 µg/plate	AMPA (> 99%)	Yes	Negative	Negative	Callander (1988b)
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537	310–5 000 µg/plate	AMPA (99.2%)	Yes	Negative	Negative	Jensen (1993a)
***N*-Acetyl-AMPA**							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	50–5 000 µg/plate	*N*-acetyl-AMPA (76%; IN-EY252)	Yes	Negative	Negative	Wagner & Klug (2007)
***N*-Acetyl-glyphosate**							
Point mutations	*S. typhimurium* TA98, 100, 1535, 1537; *E. coli* WP2 uvrA	100–5 000 µg/plate	*N*-acetyl-glyphosate sodium salt (84.3%)	Yes	Negative	Negative	Mecchi (2004)

AMPA, aminomethylphosphonic acid; GLP: good laboratory practice; N/A: not applicable; S9: 9000 × *g* supernatant fraction from induced male rat liver homogenate; –S9: without metabolic activation; +S9: with metabolic activation; UV: ultraviolet

Mammalian cells

Glyphosate and its formulation products were tested for various types of genetic damage in mammalian cells in vitro (Table 34). The results are summarized as follows. Of the four in vitro studies of gene mutation in mammalian cells induced by glyphosate or its formulation products, no increases were reported. In contrast, nine of 10 studies investigating DNA strand breaks induced by glyphosate or Roundup in mammalian cells reported positive results, 4 of 11 studies of chromosome aberrations reported positive results. For two of these (Lioi et al., 1998a,b), the effects were seen at much lower concentrations than the other studies reporting negative results. Two studies reported

negative results for polyploidy. One study of the glyphosate formulation product Herbazed (Amer et al., 2006) reported an induction of chromosome aberrations in mouse splenocytes in vitro (see further discussion of Herbazed below). Five of eight studies of micronuclei were positive, two were negative and one was equivocal; three of the positive studies required S9 whereas two did not. Of the eight studies of sister chromatid exchanges induced in peripheral blood lymphocytes, seven were positive; four were in human peripheral blood lymphocytes, two were in bovine peripheral blood lymphocytes, and one was in mouse splenocytes. Both in vitro studies of unscheduled DNA synthesis in rat hepatocytes were negative.

AMPA was negative in two studies of unscheduled DNA synthesis in isolated rat hepatocytes (Bakke, 1991; Nesslany, 2002). Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

Table 34. Summary of in vitro genotoxicity studies with glyphosate, AMPA, metabolites of AMPA and formulants in mammalian cells

End-point	Test object	Concentration	Purity	GLP (Yes/No)	Results		Reference
					−S9	+S9	
Glyphosate							
Gene mutation (*HPRT*)	CHO cells	2–25 mg/mL	Glyphosate (98%)	No	Negative	Negative	Li & Long (1988)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	0.094–5 mg/mL	Glyphosate trimesium ICIA 0224 (57.6%)	Yes	Negative	Negative	Cross (1988)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	0.52–5 mg/mL	Glyphosate (98.6%)	Yes	Negative	Negative	Jensen (1991b)
Gene mutation (*TK*)	Mouse lymphoma cells (L5178Y $TK^{\pm}$)	44–1 500 µg/mL	Glyphosate (95.6%)	Yes	Negative	Negative	Clay (1996)
Chromosomal aberrations	Mouse splenocytes	0.1–50 mmol/L	Herbazed (glyphosate, 84%)	No	Positive	N/A	Amer et al. (2006)
Chromosomal aberrations	CHO cells	4–10 µL/mL	Glyphosate trimesium SC-0224 (55.6%)	Yes	Negative	Negative	Majeska (1985)
Chromosomal aberrations	Chinese hamster cells (CHL/IU)	37.5–1 200 µg/mL	AK-01 Technical (glyphosate acid) (96.4%)	Yes	Negative	Positive	Yanagimoto (1992a)
Chromosomal aberrations	Chinese hamster lung cells	62.5–1 000 µg/mL	HR-001 (95.7%)	Yes	Negative	Negative	Matsumoto (1995)
Chromosomal aberrations	Human peripheral blood lymphocytes	33–562 µg/mL	Glyfosaat	Yes	Negative	Negative	Van de Waart (1995)
Chromosomal aberrations	Chinese hamster lung cells	39–1250 µg/mL	Glyphosate (technical grade; 95.3%)	Yes	Negative	Negative	Wright (1996)

End-point	Test object	Concentration	Purity	GLP (Yes/No)	Results –S9	Results +S9	Reference
Chromosomal aberrations	Bovine lymphocytes	17–170 µmol/L	Glyphosate	No	Positive	N/A	Lioi et al. (1998a)
Chromosomal aberrations	Human peripheral blood lymphocytes	100–1250 µg/mL	Glyphosate (95.6%)	Yes	Negative	Negative	Fox (1998)
Chromosomal aberrations	Human peripheral blood lymphocytes	5–51 µmol/L	Glyphosate (≤ 98%)	No	Positive	N/A	Lioi et al. (1998b)
Chromosomal aberrations	Human peripheral blood lymphocytes	100–4 000 µg/mL	TMS Chloride (95%) [Glyphosate trimesium]	Yes	Equivocal	Equivocal	Griffiths & Mackay (1993)
Chromosomal aberrations	Human peripheral blood lymphocytes	0.2–6 mmol/L	Glyphosate (analytical grade; 96%)	No	Negative	N/A	Manas et al. (2009a)
Micronucleus	CHO K1 cells	5–100 µg/mL	Glyphosate	No	Negative	Positive	Roustan et al. (2014)
Micronucleus	Bovine lymphocytes	28–560 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Equivocal	N/A	Piesova (2004)
Micronucleus	Bovine lymphocytes	28–560 µg/mL	Glyphosate isopropylamine salt mixture (62%)	No	Equivocal	Negative	Piesova (2005)
Micronucleus	Bovine lymphocytes	28–1 120 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Negative	N/A	Sivikova et al. (2006)
Micronucleus	Human peripheral blood lymphocytes	0.5–580 µg/mL	Glyphosate (technical grade: 98%)	No	Negative	Positive	Mladinic et al. (2009)
Micronucleus	Human epithelial cancer cell line TR146	10–20 mg/L	Glyphosate (95%)	No	Positive	N/A	Koller et al. (2012)
Micronucleus	Human epithelial cancer cell line TR146	10–20 mg/L	Roundup	No	Positive	N/A	Koller et al. (2012)
Micronucleus	CHO K1 cells	5–100 µg/mL	Glyphosate	No	Negative	Positive	Roustan et al. (2014)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM5757	75 mmol/L	Glyphosate (98.4%)	No	Negative alone; positive in presence of H_2O_2	N/A	Lueken et al. (2004)

End-point	Test object	Concentration	Purity	GLP (Yes/No)	Results –S9	Results +S9	Reference
DNA strand breaks (Comet assay)	Human fibrosarcoma cell line HT1080	4.5–6.5 nmol/L	Glyphosate (technical grade)	No	Positive	N/A	Lopez et al. (2005)
DNA strand breaks (Comet assay)	Human fibroblast cell line GM38	4.5–6.5 nmol/L	Glyphosate (technical grade)	No	Positive	N/A	Lopez et al. (2005)
DNA strand breaks (Comet assay)	Human liver HepG2 cell line	1–10 ppm	Roundup (R400)	No	Positive	N/A	Gasnier et al. (2009)
DNA strand breaks (Comet assay)	Human Hep2 cell line	3–7.5 mmol/L	Glyphosate (analytical grade; 96%)	No	Positive	N/A	Manas et al. (2009a)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	0.5–580 µg/mL	Glyphosate (technical grade; 98%)	No	Positive	Positive	Mladinic et al. (2009)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	10–2 000 mg/L	Glyphosate (95%)	No	Positive	N/A	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human epithelial cancer cell line TR146	10–2 000 mg/L	Roundup	No	Positive	N/A	Koller et al. (2012)
DNA strand breaks (Comet assay)	Human peripheral blood lymphocytes	0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	N/A	Alvarez-Moya et al. (2014)
DNA strand breaks	Mouse spermatogonia	60–180 mg/L	Glyphosate		Positive	N/A	Ming et al. (2014)
Sister chromatid exchange	Mouse splenocytes	0.1–50 mmol/L	Herbazed (glyphosate, 84%) .	No	Positive	N/A	Amer et al. (2006)
Sister chromatid exchange	CHO cells	4–10 µL/mL	Glyphosate trimesium SC-0224 (55.6%)	Yes	Negative	Negative	Majeska (1985)
Sister chromatid exchange	Bovine lymphocytes	28–1 120 µmol/L	Glyphosate isopropylamine salt mixture (62%)	No	Positive	N/A	Sivikova et al. (2006)
Sister chromatid exchange	Bovine lymphocytes	17–170 µmol/L	Glyphosate	No	Positive	N/A	Lioi et al. (1998a)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.25–25 mg/mL	Roundup	No	Positive	N/A	Vigfusson & Vyse (1980)
Sister chromatid exchange	Human peripheral blood lymphocytes	0.33–6 µg/mL	Glyphosate (analytical grade; 99.9%)	No	Positive	N/A	Bolognesi et al. (1997a)

End-point	Test object	Concentration	Purity	GLP (Yes/No)	Results		Reference
					–S9	+S9	
Sister chromatid exchange	Human peripheral blood lymphocytes	0.1–0.33 µg/mL	Roundup (30.4% glyphosate)	No	Positive	N/A	Bolognesi et al. (1997a)
Sister chromatid exchange	Human peripheral blood lymphocytes	5–51 µmol/L	Glyphosate (≥ 98%)	No	Positive	N/A	Lioi et al. (1998b)
Unscheduled DNA synthesis	Rat hepatocytes	0.000 012 5–0.125 mg/mL	Glyphosate (98%)	No	Negative	N/A	Li & Long (1988)
Unscheduled DNA synthesis	Rat hepatocytes	0.2–111.7 mmol/L	Glyphosate (≥ 98%)	Yes	Negative	N/A	Rossberger (1994)
AMPA							
Gene mutation	Mouse lymphoma cells (L5871Y)	0.31–5.0 mg/mL	99.2%	Yes	Negative	Negative	Jensen (1993b)
Chromosomal aberrations	Human peripheral lymphocytes	0.9–1.8 mmol/L	99%	No	Weak positive	N/A	Manas et al. (2009b)
Micronucleus	CHO K1 cells	0.005–0.1 µg/L	AMPA (purity unspecified)	N/S	Positive	Positive	Roustan et al. (2014)
Micronucleus	CHO K1 cells	5–100	Glyphosate + AMPA	N/S	Negative	Negative	Roustan et al. (2014)
DNA strand breaks (Comet assay)	Human Hep2 cell line	2.5–7.5 mmol/L	99%	No	Positive	N/A	Manas et al. (2009b)
Unscheduled DNA synthesis	Rat hepatocytes	5–2 500 µg/mL	94.4%	N/S	Negative	N/A	Bakke (1991)
Unscheduled DNA synthesis	Rat hepatocytes	0.078–10 mmol/L	99.9%	N/S	Negative	N/A	Neslany (2002)
***N*-Acetyl-AMPA**							
Chromosomal aberrations	Human peripheral blood lymphocytes	191–1 530 µg/mL	76%; IN-EY252	Yes	Negative	Negative	Gudi & Rao (2007)
Gene mutation (*HPRT*)	CHO cells	100–1 531 µg/mL (active ingredient, adjusted for purity)	72%; IN-EY252	Yes	Negative	Negative	Glatt (2007)
***N*-Acetyl-glyphosate**							
Gene mutation (*HPRT*)	CHO cells	250–2 091 µg/mL (active ingredient, adjusted for purity)	*N*-acetyl-glyphosate sodium salt (63%)	Yes	Negative	Negative	Glatt (2006)

End-point	Test object	Concentration	Purity	GLP (Yes/No)	Results –S9	Results +S9	Reference
Chromosomal aberrations	CHO cells	960–2 800 µg/mL	*N*-acetyl-glyphosate sodium salt (84.3%)	Yes	Negative	N/A	Murli (2004)

AMPA: aminomethylphosphonic acid; CHO: Chinese hamster ovary; GLP: good laboratory practice; HepG2: hepatocellular carcinoma; Hep2: epidermoid cancer; HPRT: hypoxanthine-guanine phosphoribosyltransferase; N/A: not applicable; N/S: not stated; ppm: parts per million; S9: 9000 × *g* supernatant fraction from male rat liver homogenate; –S9: without metabolic activation; +S9: with metabolic activation; TK: thymidine kinase

(b) In vivo studies

Mammalian studies

Oral route

Thirty-three in vivo genotoxicity studies assessed the effect of orally administered glyphosate or its formulation products on rodents (29 in mice and four in rats). The end-points investigated included chromosomal alterations, micronuclei, sister chromatid exchanges, unscheduled DNA synthesis and dominant lethal mutations (Table 35). Fourteen of the studies were conducted using glyphosate (≥ 90% pure) with the remainder involving formulation products or less pure forms of glyphosate. The results were negative for 29 of the 33 studies. The majority of the studies were of good or acceptable quality, and included sponsored GLP studies conducted in compliance with OECD Guideline 474.

The four positive studies are briefly described here. A twofold statistically significant increase in micronucleus frequency was reported by Suresh (1993a) in female (but not male) mice treated with two 5000 mg/kg doses of glyphosate. (The JMPR committee noted that this dose exceeds the limit dose of 2000 mg/kg recommended by the OECD [2014] and the International Conference on Harmonisation of Technical Requirements for Pharmaceuticals for Human Use [2011]. The micronucleus frequencies in the concurrent control were also higher than normal, and historical control frequencies for the lab were not provided. In addition, a study published the following year by the same group using the same doses of glyphosate did not see an increase in glyphosate-induced chromosome aberrations.] The three other positive studies were described in one article, a study published by Amer et al. (2006). In this article, positive results in both bone marrow cells and spermatocytes were reported after the administration of seven or more doses of a glyphosate formulation product called Herbazed (other positive results from that study are presented below). In contrast, in a repeated-dose study conducted by the United States National Toxicology Program (Chan & Mahler, 1992), increases in micronuclei were not seen in bone marrow erythrocytes of male and female mice administered glyphosate in the diet for 13 weeks. In another repeated-dose study, increases in chromosome aberrations were not seen in rat bone marrow cells harvested after 5 days of treatment with glyphosate trimesium (Matheson, 1982). Amer et al. (2006) also reported an increase in sister chromatid exchanges in mouse bone marrow cells after a single Herbazed dose.

Intraperitoneal injection

The JMPR committee concluded that genotoxic effects in animals treated with glyphosate or its formulation products by intraperitoneal injection were of limited value in assessing risks due to low-level dietary exposure. The following description of results is presented for completeness.

Twenty-one studies of micronuclei and chromosomal alterations were performed in the bone marrow cells of rodents administered glyphosate or its formulation products by intraperitoneal injection. Positive results were reported in approximately one third of the studies and negative/equivocal results for the remaining two thirds. The positive studies were reported in articles by four groups (Bolognesi et al., 1997; Prasad et al., 2009, Manas et al., 2009a; Rodrigues et al.,

2011) and involved the administration of both glyphosate and its formulation products. The Rodrigues et al. (2011) and Prasad et al. (2009) studies reported increases in micronuclei at doses (≥ 0.75 mg/kg bw and ≥ 25 mg/kg bw of Roundup, respectively) that were considerably lower than those reported as negative by other investigators (e.g. Jensen, 1991c [5000 mg/kg bw] and Kier, Flowers & Huffman, 1992 [850–3400 mg/kg bw]). When positive results were seen and when a direct comparison could be made, the formulation product was more potent than glyphosate itself (Bolognesi et al., 1997). Positive results in mouse spermatocytes were also reported with administration of 50 mg/kg bw of the glyphosate formulation product Herbazed for 5 days or more (but not 1 or 3 days) (Amer et al., 2006).

Increases in DNA strand breaks in the liver and kidney of mice were reported for both glyphosate and Roundup by Bolognesi et al. (1997). Heydens et al. (2008) conducted a follow-up study using the same Roundup formulation and reported that significant toxicity occurred in the liver and kidney when dosing was by intraperitoneal injection. They postulated that the DNA damage reported by Bolognesi et al. (1997) was likely a secondary effect of toxicity.

Bolognesi and colleagues (Peluso et al., 1998) also reported an increase in DNA adducts in mouse liver and kidney by the sensitive but nonspecific ^{32}P-postlabelling method following intraperitoneal administration of Roundup, but not glyphosate. They attributed the adducts to an unknown component of the herbicide mixture. This same group of investigators reported that intraperitoneal administration of glyphosate and Roundup resulted in an increase in 8-hydroxy-2'-deoxyguanosine (8-OHdG) DNA adducts in the liver (glyphosate) and kidney (Roundup). A follow-up study on Roundup by Heydens et al. (2008) was unable to replicate the 8-OHdG adduct results.

Table 35. Summary of in vivo genotoxicity studies with glyphosate, glyphosate formulation products and AMPA and their metabolites in mammalian species

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Glyphosate						
Oral administration						
Dominant lethal test	Mouse fetuses and resorptions	200–2 000 mg/kg	Glyphosate (98.7%)	Yes	Negative	Rodwell (1980)
Chromosomal aberrations	Mouse bone marrow cells	50–5 000 mg/kg on 2 days	Glyphosate (96.8%)	Yes	Negative in males and females	Suresh (1994)
Chromosomal aberrations	Mouse bone marrow cells	1 080 mg/kg bw	Roundup (> 90% purity)	No	Negative in males	Dimitrov et al. (2006)
Chromosomal aberrations	Mouse bone marrow cells	50 and 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse spermatocytes	50 and 100 mg/kg bw (daily up to 21 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Rat bone marrow cells	21–188 mg/kg	Glyphosate trimesium SC-0224 (58.5%)	No	Negative in males at all time points up to 5 days of exposure	Majeska (1982b)
Micronucleus	Mouse bone marrow erythrocytes	400–1 100 mg/kg	Glyphosate trimesium SC-0224 (55.3%)	Yes	Negative in males and females	Majeska (1986)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	3–50 mg/kg in the diet	Glyphosate (98.6%)	No	Negative in males and females	Chan & Mahler (1992)
Micronucleus	Mouse bone marrow erythrocytes	50–5 000 mg/kg bw; administered twice	Glyphosate (96.8%)	Yes	Negative for males; weak positive / equivocal for females at highest dose	Suresh (1993a)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw	Glyphosate (95.6%)	Yes	Negative in males and females	Fox & Mackay (1996)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate potassium salt (49% glyphosate acid by analysis) [indicated 59.3% in text]	Yes	Negative in males	Jones (1999)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78634 (65.2% glyphosate)	Yes	Negative in males	Erexson (2003)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	AK-01 Technical (99.1%)	Yes	Negative in males	Inoue (2004)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate technical (97.73%)	Yes	Negative in males and females	Honarvar (2005)
Micronucleus	Mouse bone marrow erythrocytes	1 080 mg/kg bw	Roundup (> 90% purity)	No	Negative in males	Dimitrov et al. (2006)
Micronucleus	Mouse bone marrow erythrocytes	8–30 mg/kg bw	Glyphosate technical Helm (≥ 95%)	Yes	Negative / equivocal in males	Zoriki Hosomi (2007)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate (99.1%)	Yes	Negative in males	Honarvar (2008)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 79864 (38.7% glyphosate)	Yes	Negative in males	Xu (2008a)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76171 (31.1% glyphosate)	Yes	Negative in males	Xu (2008b)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76313 (30.9% glyphosate)	Yes	Negative in males	Xu (2008c)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate (A17035A) (280 g/L)	Yes	Negative in males	Negro Silva (2009)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 79991 (71.6% glyphosate)	Yes	Negative in males	Xu (2009a)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 76138 (38.5% glyphosate)	Yes	Negative in males	Xu (2009b)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78910 (30.3% glyphosate)	Yes	Negative in males	Xu (2010) [amended version of Erexson (200 6)]
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	TROP M (Glyphosate 480) (358.4 g/L glyphosate acid: 483.6 g/L glyphosate isopropylamine salt)	Yes	Negative in males and females	Flügge (2010)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate soluble liquid concentrate (A13013Z) (500 g/L)	Yes	Negative in males	Negro Silva (2011)
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw	MON 78239 (36.6% glyphosate)	Yes	Negative in males	Xu (2011) [amended version of Erexson (200 3)]
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate (96.3%)	Yes	Negative in males	Roth (2012)
Micronucleus	Mouse bone marrow erythrocytes	2 000 mg/kg bw	Glyphosate TGAI (98.9%)	Yes	Negative in males	Patel (2012)
Micronucleus	Rat bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate technical grade (98.8%)	Yes	Negative in males and females	Flügge (2009 b)
Micronucleus	Rat bone marrow erythrocytes	500–2 000 mg/kg bw	Glyphosate 75.5 DF (69.1% glyphosate)	Yes	Negative in males and females	Flügge (2010)
Unscheduled DNA synthesis	Rat liver hepatocytes	150–600 mg/kg bw	Glyphosate trimesium ICIA0224 (57.6%)	Yes	Negative in males	Kennelly (19 90)
Sister chromatid exchange	Mouse bone marrow cells	50–200 mg/kg bw	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Intraperitoneal administration						
Chromosomal aberrations	Rat bone marrow cells	1 000 mg/kg bw	Glyphosate (98%)	No	Negative in males and females	Li & Long (1988)
Chromosomal aberrations	Mouse bone marrow cells	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse spermatocytes	50 mg/kg bw (daily up to 5 days)	Herbazed (glyphosate, 84%)	No	Positive in males	Amer et al. (2006)
Chromosomal aberrations	Mouse bone marrow cells	25 and 50 mg/kg bw	Roundup (> 41%)	No	Positive in males	Prasad et al. (2009)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw	Glyphosate (98.6%)	Yes	Negative in males and females	Jensen (1991 c)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	850–3 400 mg/kg bw	Rodeo formulation (40%)	Yes	Negative in males and females	Kier, Flowers & Huffman (1992)
Micronucleus	Mouse bone marrow erythrocytes	100–200 mg/kg bw	Glyphosate isopropylamine salt	No	Negative in combined males and females	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	133 and 200 mg/kg bw as glyphosate isopropylamine salt	Roundup (480 g/L)	No	Negative in combined males and females	Rank et al. (1993)
Micronucleus	Mouse bone marrow erythrocytes	68–206 mg/kg bw	Glifos (360 g/L glyphosate)	No	Negative in males and females	Zaccaria (1996)
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	450 mg/kg bw; 135 mg/kg as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
Micronucleus	Mouse bone marrow erythrocytes	188–563 mg/kg bw	Glyphosate technical Nufarm (95%)	Yes	Negative in combined males and females	Carvalho Marques (1999)
Micronucleus	Mouse bone marrow erythrocytes	300 mg/kg bw	Glyphosate technical grade	No	Negative in males	Chruscielska et al. (2000b)
Micronucleus	Mouse bone marrow erythrocytes	90 mg/kg bw	Glyphosate formulation Perzocyd 10 soluble liquid concentrate	No	Negative in males	Chruscielska et al. (2000b)
Micronucleus	Mouse bone marrow erythrocytes	50–200 mg/kg bw	Glyphosate (Roundup 69)	No	Negative (sex not specified)	Nascimento & Grisolia (2000)
Micronucleus	Mouse bone marrow erythrocytes	1 008–3 024 mg/kg bw	Glifosato IPA Technico Nufar; glyphosate isopropylamine salt (613 g/kg salt equivalent)	Yes	Negative in males and females	Gava (2000)
Micronucleus	Mouse bone marrow erythrocytes	50–200 mg/kg bw	Roundup (480 g/L)	No	Negative in combined males and females	Grisolia (2002)
Micronucleus	Mouse bone marrow erythrocytes	150–600 mg/kg bw	Glyphosate technical grade (95.7%)	Yes	Negative/equivocal in males	Durward (2006)
Micronucleus	Mouse bone marrow erythrocytes	15.6–62.5 mg/kg bw	Glyphosate technical grade (98%)	Yes	Negative in males and females	Costa (2008)
Micronucleus	Mouse bone marrow erythrocytes	25 and 50 mg/kg bw	Roundup (> 41%)	No	Positive in males	Prasad et al. (2009)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Micronucleus	Mouse bone marrow erythrocytes	100–400 mg/kg bw	Glyphosate (analytical grade; 96%)	No	Positive in combined males and females	Manas et al. (2009a)
Micronucleus	Mouse bone marrow erythrocytes	0.148–1.28 mg/kg bw	Roundup	No	Positive (sex not specified)	Rodrigues et al. (2011)
DNA strand breaks	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
DNA strand breaks	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
DNA adducts by ^{32}P-postlabelling	Liver and kidney of mice	130 and 270 mg/kg	Glyphosate isopropylammonium salt	No	Negative in combined males and females	Peluso et al. (1998)
DNA adducts by ^{32}P-postlabelling	Liver and kidney of mice	400–600 mg/kg	Roundup (30.4%)	No	Positive in combined males and females	Peluso et al. (1998)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	300 mg/kg bw	Glyphosate (analytical grade; 99.9%)	No	Positive in males	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	900 mg/kg bw; 270 mg/kg bw as glyphosate	Roundup (30.4%)	No	Positive in males	Bolognesi et al. (1997)
Oxidative DNA adducts (8-OHdG)	Liver and kidney of mice	600 and 900 mg/kg bw	Glyphosate formulation (30.4%)	No	Negative in males	Heydens et al. (2008)
AMPA						
Micronucleus	Mouse bone marrow erythrocytes	100–1 000 mg/kg bw IP	AMPA (94.4%)	Yes	Negative in males and females	Kier & Stegeman (1993)
Micronucleus	Mouse bone marrow erythrocytes	5 000 mg/kg bw oral route	AMPA (99.2%)	Yes	Negative in males and females	Jensen (1993c)
Micronucleus	Mouse bone marrow erythrocytes	200–400 mg/kg bw IP	AMPA (99%)	No	Positive	Manas et al. (2009b)
***N*-acetyl-AMPA**						
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw (active ingredient, adjusted for purity) oral route	*N*-acetyl-AMPA (72%; IN-EY252)	Yes	Negative in males and females	Donner (2007)

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
***N*-Acetyl-glyphosate**						
Micronucleus	Mouse bone marrow erythrocytes	500–2 000 mg/kg bw (active ingredient, adjusted for purity) oral route	*N*-acetyl-glyphosate (63%; IN-MCX20)	Yes	Negative in males and females	Donner (2006)
Other related chemicals						
Chromosomal aberrations	Mouse bone marrow cells	10 and 100 mg/kg bw	Series of α-aminophosphonic acids	No	Positive	Naydenova et al. (2007)

AMPA: aminomethylphosphonic acid; bw: body weight; GLP: Good laboratory practice; IP: intraperitoneal; N/S: not stated; 8-OHdG: 8-hydroxy-2'-deoxyguanosine

(c) Non-traditional tests or tests in phylogenetically distant organisms

The results of genotoxicity studies in phylogenetically distant organisms or using non-traditional and generally non-validated assays are presented in Appendix 1. Studies were performed both in vitro and in vivo with most of the tests measuring DNA strand breakage or micronucleus formation. Approximately two thirds of these studies reported positive results. Mixed positive and negative results were seen in mutation studies in *Drosophila*. The reason for the differences in response between these species and those seen in mammals orally administered glyphosate is not known. Surfactants and other components of the glyphosate formulation products have been reported to be toxic to fish and other species, and this may contribute to the observed differences in test results (Howe et al., 2004; Guilherme et al., 2012a; Navarro & Martinez, 2014). For example, the surfactant polyoxyethylene amine, a common component in glyphosate formulations, was shown to induce several indices of toxicity in the neotropical fish *Prochilodus lineatus* at all of the doses tested (Navarro & Martinez, 2014).

(d) Human biomonitoring studies

The association between exposure to glyphosate and increase in micronucleus frequencies in peripheral blood lymphocytes, as well as the persistence of any effects over time, was evaluated over several months in individuals living in three areas of Colombia where glyphosate formulations were aerially sprayed over illicit and legal crops (Bolognesi et al., 2009). Significant increases in micronucleus frequencies were observed several days after spraying, but these increases did not correlate with glyphosate spray rates. Over time, the induced micronucleus frequencies decreased among the people in one area, remained the same among those in another, and increased among those in the third. In addition, in all three communities, the micronucleus frequencies of individuals who reported being directly exposed to glyphosate did not differ from those who reported no glyphosate exposure.

The JMPR committee reviewed the studies and considered the results to be inconclusive or equivocal. It noted that the micronucleus frequencies in the reference population were unusually low and that the frequencies within the glyphosate-exposed communities fall well within the normal range for non-exposed individuals (Bonassi et al., 2001). The results were considered to be inadequate to reach a conclusion on the potential chromosome-damaging properties of glyphosate in humans.

Another study used the Comet assay to determine the frequency of DNA strand breakage in the peripheral blood lymphocytes of individuals living in an Ecuadorian community within 3 kilometres of where glyphosate was sprayed. The frequency of DNA strand breakage was reported to be significantly higher than that of individuals living in a community 80 kilometres away where

glyphosate was not used (Paz-y-Mino et al., 2007). The samples were collected from exposed individuals 2 weeks to 2 months after the spraying had occurred. In reviewing the study, the JMPR committee noted that the study had some major deficiencies; the blood samples of the two groups were collected and processed at different times, a key consideration for an assay that is highly prone to technical artefacts during sample preparation. In addition, the two populations were located at considerable distance from each other, the background frequencies of DNA breakage in these communities was not known, and the median DNA migration values were identical for 20 of the 21 subjects in the control population, a result that was considered to be highly unusual.

The JMPR committee concluded that the study was inconclusive as problems with study design severely limit the conclusions that can be drawn.

In a follow-up study by the same authors, the frequency of structural chromosomal aberrations in peripheral blood lymphocytes was measured in the study population that two years previously had been exposed to glyphosate; the frequencies were found to be normal (Paz-y-Mino, 2011). The study results were considered to be negative but minimally informative as many types of chromosome alterations do not persist for extended periods of time.

In another study, the levels of 8-OHdG, a lesion formed from oxidative damage to DNA, were measured in the peripheral blood lymphocytes of workers spraying glyphosate (Koureas et al., 2014). A modestly elevated but statistically nonsignificant increase was reported.

Summaries of these biomonitoring studies are shown in Table 36.

Table 36. Summary of human biomonitoring studies

End-point	Test object	Concentration	Purity	GLP (Yes/ No)	Results	Reference
Structural chromosomal aberrations	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Colombia	Glyphosate-containing mixture	No	Negative	Paz-y-Mino et al. (2011)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Narino, Colombia	Herbicide mixtures containing glyphosate and adjuvant	No	Equivocal/inconclusive	Bolognesi et al. (2009)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Putumayo, Colombia	Herbicide mixtures containing glyphosate and adjuvant	No	Equivocal / inconclusive	Bolognesi et al. (2009)
Micronucleus	Human peripheral blood lymphocytes	Aerial spraying, Valle del Cuaca, Colombia	Roundup 47	No	Equivocal / inconclusive	Bolognesi et al. (2009)
DNA strand breaks/Comet	Human peripheral blood cells	Aerial spraying, Ecuadorian region bordering Colombia	Roundup Ultra (44%)	No	Equivocal/ inconclusive	Paz-y-Mino et al. (2007)
DNA adducts (8-OHdG)	Human peripheral blood cells	Pesticide applicators	Glyphosate	No	Negative	Koureas et al. (2014)

8-OHdG: 8-hydroxy-2'-deoxyguanosine

(e) Mechanistic considerations

Neither glyphosate nor its metabolites possess the chemical structural motifs commonly associated with mutagenesis or carcinogenesis (Ashby et al., 1989; Kier and Kirkland, 2013). However, one study investigating the effects of a series of α-aminophosphonic acids with structural similarities to glyphosate, reported moderate clastogenic activity in the mouse bone marrow chromosome aberration test when administered by intraperitoneal injection (Naydenova et al., 2007). In contrast, glyphosate bioassay results in 620 assays screening biological activity including cytotoxicity are reported in PubChem (accessed 20 April 2016). Positive results were seen only in 21 of the 620 assay reports, the majority of which appear to be closely related to glyphosate's herbicidal mechanism of action in plants. The few other positives involved protein-ligand binding and inhibition of the metabolic enzyme CYP71B1. These results indicate that, at the concentrations tested and at the end-points examined, glyphosate had few off-target molecular or cellular effects.

Summary:

The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg bw by the oral route, the route most relevant to human dietary exposure, was not associated with an increase in chromosome alterations or other types of genetic damage. The majority of the in vivo studies were conducted in rodents, a model considered physiologically relevant for assessing genotoxic risks to humans. The genotoxic effects reported to occur in vitro or in phylogenetically distant organisms have not been observed in vivo in appropriately treated mammalian models.

When administered by intraperitoneal injection, mixed, largely negative, results have been reported in studies of chromosomal damage of glyphosate, its formulation products and metabolites. Mixed, and somewhat contradictory, results have been reported in the few studies (all conducted by intraperitoneal injection) that have investigated DNA adducts induced by glyphosate or Roundup. Results obtained by this route of administration are considered to have limited relevance when estimating risks from human dietary exposure.

The positive results reported by Amer et al. (2006) using both oral and intraperitoneal routes of administration appear anomalous, and may have been due to impurities or other components within the Herbazed formulation product.

Biomonitoring studies of DNA and chromosomal alterations in humans conducted in five to six communities by several investigators found equivocal associations between glyphosate exposure and genetic damage.

2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

In a non-GLP three-generation reproduction study, glyphosate (purity 100%) was fed in the diet to 12 male and 24 female CD rats at concentrations of 0, 3, 10 or 30 mg/kg bw per day starting 63 days prior to mating. Each male was mated with two females. The first litters (F_{1A}, F_{2A}, and F_{3A}) from each mating were raised to weaning and then terminated. Second matings (F_{1B} and F_{2B}) were selected to become parents for subsequent generations or to undergo complete gross necropsy (F_{3B}). Tissues were also evaluated microscopically (10/sex/group) from the control and high-dose parental animals for all generations and F_{3B} offspring.

Analytical results demonstrated that glyphosate was stable and homogenously distributed in the diet. Analysis of various batches showed an average of 98.0 (± 6.8)% of the target concentration. No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or

duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

No adverse effects were noted in the study. The NOAEL for parental, reproductive and offspring toxicity was 30 mg/kg bw per day, the highest dose tested (Schroeder & Hogan, 1981).

In a two-generation reproduction study, glyphosate (purity 97.67%) was administered to Sprague Dawley rats (30/sex per dose) in the diet at concentrations of 0, 2000, 10 000 or 30 000 ppm (equal to 0, 132, 666 and 1983 mg/kg bw per day for males and 0, 160, 777 and 2322 mg/kg bw per day for females). After approximately 11 weeks of treatment, pairs of animals within each dose group were mated on a 1:1 basis to produce the F_1 litters. At weaning, 30 of these F_1 generation rats (referred to as F_{1A} in study report) per sex per dose were similarly exposed (approximately 14 weeks) and mated twice to produce F_{2A} and F_{2B} generations. On day 4 postpartum, litters were standardized (four males and four females when possible). Offspring not selected for mating, F_{2A} and F_{2B} pups, and adult females which had littered were terminated on or after day 21 of lactation. Adult males were terminated after mating. Organs were retained from all parental animals and one pup per sex per litter from F_{2A} and F_{2B}. Tissues from control and high-dose animals were examined microscopically.

The stability and homogeneity of glyphosate in the diet were acceptable. Analytical concentrations were, on the average, 95–96.7% of target levels. No treatment-related adverse effects were observed on mortality, feed consumption, organ weights or histopathological changes for parental animals of either generation. The incidence of soft stools was increased for high-dose adult animals in both generations (Table 37). Reduced body weights were noted in parental animals of both generations at termination: body weights were approximately 8–10% lower than controls for the F_0 generation and 10–13% lower than controls in the F_1 generation (Table 38).

No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Compared to the controls, there was a slight reduction in average litter size for F_0 dams in the highest dose group; an even smaller difference was noted after the first F_1 mating. However, the slight reduction in average litter size was not statistically significant. The F_{1a} adults were re-mated to produce the F_{2b} generation. There was no dose-related decrease in litter size in this second mating. Since the reductions in litter size were neither statistically significant nor consistently observed in all generations, the relationship to treatment could not be conclusively established. Therefore, it was concluded that litter size and viability were not affected by treatment.

No adverse effects were noted for offspring body weights or development. Statistically significant differences in pup body weights compared to controls were observed at mid and high dose, but these differences were small and within biologically variability.

Table 37. Soft stools in two successive generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 ppm	**2 000 ppm**	**10 000 ppm**	**30 000 ppm**
F_0 – males				
No. of animals	0	0	0	30/30
No. of occurrences	0	0	0	457
F_0 – females				
No. of animals	0	0	0	22/30
No. of occurrences	0	0	0	116
F_1 – males				
No. of animals	0	0	1/30	30/30
No. of occurrences	0	0	1/30	698
F_1 – females				
No. of animals	0	0	0	29/30

	Incidence per dietary concentration of glyphosate			
	0 ppm	2 000 ppm	10 000 ppm	30 000 ppm
Number of occurrences	0	0	0	537

ppm: parts per million; F_0: parental generation; F_1: first filial generation; No.: number

Results presented as number of animals with soft stools / number of animals examined.

Source: Reyna (1990)

Table 38. Terminal body weights in two successive generations of parental rats administered glyphosate

	Weight per dietary concentration of glyphosate			
	0	2 000 ppm	10 000 ppm	30 000 ppm
F_0				
Males	549.6 ± 46.8	550.2 ± 80.7	540.0 ± 58.1	503.5 ± 45.7 (↓8%)
Females	296.3 ± 23.6	290.6 ± 19.5	290.7 ± 25.4	265.9 ± 15.4 (↓10%)
F_1				
Males	625.0 ± 53.1	632.1 ± 74.6	591.0 ± 70.1	543.4 ± 58.1 (↓13%)
Females	316.2 ± 37.4	313.7 ± 30.5	312.4 ± 26.7	284.7 ± 18.4 (↓10%)

ppm: parts per million; F_0: parental generation; F_1: first filial generation; no.: number; ↓: decrease

Results presented as mean weight in grams ± standard deviations, with per cent change relative to controls in parentheses for the high-dose group only.

Source: Reyna (1990)

The NOAEL for parental toxicity was 10 000 ppm (equal to 666 mg/kg bw per day) based on decreased body weights and increased incidence of soft stools in rats at 30 000 ppm. As there were no effects on reproductive parameters or offspring measurements, the NOAEL for reproductive and offspring toxicity was 30 000 ppm (equal to 1983 mg/kg bw per day (Reyna, 1990).

In a two-generation reproduction study, groups of 28 male and 28 female Crl:CD(SD)BR VAF/Plus rats (aged 6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity 99.2%) at concentrations of 0, 1000, 3000 or 10 000 ppm (equal to 0, 66.4, 196.8 and 668.1 mg/kg bw per day for males and 0, 75.3, 226.0 and 752.3 mg/kg bw per day for females) for 70 days before their first mating and until termination. Each generation was mated twice, changing partners for the second mating and avoiding sister/brother matings throughout. On postnatal day 4, litters were standardized (four males and four females, when possible). The remaining pups and those not selected for mating were terminated and underwent gross pathological examinations. Treatment was continued for parental animals until day 21 of weaning of the second litter when animals were terminated for organ weighing, gross pathological examination and histopathological examination. Initial histopathological examinations were performed in the control and highest dose groups. Other dose groups were analysed when an effect was seen in a tissue at the highest dose.

No treatment-related adverse effects on mortality, clinical signs, body weights, feed consumption, feed efficiency or organ weights were observed for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

Treatment-related histopathological changes were found in the parotid salivary gland of both sexes and submaxillary salivary gland of females in both generations (Table 39). The changes were

described as hypertrophy of acinar cells with prominent granular cytoplasm (minimal severity). Increased incidence of the effects was observed at the highest dose tested.

Table 39. Cellular alterations in salivary glands of two successive generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate							
	Males				Females			
Site of cellular alteration	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm
F_0								
Parotid gland	2/27	2/28	3/28	12/26	0/28	2/27	5/28	17/28
Submaxillary gland	0/27	–	–	0/26	0/28	1/27	4/28	14/28
F_1								
Parotid gland	1/24	0/24	4/23	11/23	0/24	0	4/24	9/23
Submaxillary gland	0/24	–	–	0	0/24	0	0/24	3/23

ppm: parts per million; F_0: parental generation; F_1: first filial generation; –: not examined.

Initial histopathological examinations were performed in the control and highest dose groups. Other dose groups were analysed when an effect was seen at the highest dose.

Results presented as number of animals with hypertrophy of acinar cells with prominent granular cytoplasm / number of animals examined.

Source: Brooker et al. (1992)

The NOAEL for parental toxicity was 3000 ppm (equal to 196.8 mg/kg bw per day, based on increased incidence of histopathological effects observed in the parotid (males and females) and submaxillary (females only) salivary glands in both generations of rats at 10 000 ppm (equal to 668.1 mg/kg bw per day). As there were no effects on reproductive parameters or offspring measurements, the NOAEL for reproductive and offspring toxicity of glyphosate in rats is 10 000 ppm (equal to 668.1 mg/kg bw per day) (Brooker et al., 1992).

In a two-generation reproduction study, glyphosate (purity 96.8%) was administered to Wistar (30 rats/sex per dose) in the diet at concentrations of 0, 100, 1000 or 10 000 ppm (equivalent to 0, 6.6, 66.0 and 660 mg/kg bw per day) for two successive generations with one litter per generation. The mean daily intake of glyphosate was not reported for all dietary levels; however, the low dose of 100 ppm corresponds to an average of 7.7 mg/kg bw per day according to the original study report. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. On day 4 postpartum, litters were standardized (four males and four females, if possible). At weaning, 30 males and 30 females from each dose group were selected to produce the F_1 generation; these rats were dosed for at least 10 weeks and paired within their dose group to produce F_2 litters. All parental animals, non-selected pups from F_1 and all pups from F_2 were necropsied. Only parental tissue was collected.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. No adverse effects were noted for offspring body weights or development.

As no adverse effects were noted in the study, the NOAEL for parental, reproductive and offspring toxicity in rats was 10 000 ppm (equivalent to 660 mg/kg bw per day), the highest dose tested (Suresh, 1993b).

In a two-generation reproduction study, glyphosate (purity 94.61%) was administered to 24 Crl:CD(SD) rats/sex per dose at concentrations of 0, 1200, 6000 and 30 000 ppm (equal to 0, 83.6, 417 and 2150 mg/kg bw per day for males and 0, 96.9, 485 and 2532 mg/kg bw per day for females) for two successive generations with one litter per generation. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. On day 4 postpartum, litters were standardized (four males and four females, if possible). At weaning, 24 males and 24 females from each dose group were selected to produce the F_1 generation. Unselected offspring were terminated and underwent gross necropsy. The offspring selected for the F_1 generation were dosed for at least 10 weeks and paired within dose group to produce F_2 litters. At weaning, parental animals and their offspring were terminated and examined macroscopically. Organs were taken from all parental animals for weights and histopathological examination. For offspring, the same organs were taken from one animal per sex per litter at random. The overall calculated mean daily intake of glyphosate was 0, 84, 417 and 2150 mg/kg bw per day for F_0 males; 0, 97, 485 and 2532 mg/kg bw per day for F_0 females; 0, 92, 458 and 2411 mg/kg bw per day for F_1 males; and 0, 105, 530 and 2760 mg/kg bw per day for F_1 females.

There were no treatment-related adverse effects on mortality, body weights, feed consumption, feed efficiency or histopathological changes for parental animals of either generation. The incidence of loose stools was increased for high-dose parental animals in both generations (Table 40). In addition, the incidences of caecum distension were increased in high-dose parental animals in both generations (Table 41). Although increases in liver and kidney weights were noted in the high-dose group, these changes were not considered adverse given the magnitude of the change and/or lack of corresponding histopathological changes in these organs.

Table 40. Loose stools in two generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate											
	Pre-mating				Mating/gestation				Lactation/post-weaning			
	0 ppm	1 200 ppm	6 000 ppm	30 000 ppm	0 ppm	1 200 ppm	6 000 ppm	30 000 ppm	0 ppm	1 200 ppm	6 000 ppm	30 000 ppm
F_0												
M	0/24	0/24	0/24	3/24	0/23	0/24	0/24	2/24	N/A	N/A	N/A	N/A
F	0/24	0/24	0/24	1/24	0/24	0/24	0/24	0/24	0/24	0/24	0/24	6/24
F_1												
M	0/24	0/24	0/24	13/24	0/23	0/24	0/23	0/24	N/A	N/A	N/A	N/A
F	0/24	0/24	0/24	4/24	0/23	0/23	0/21	0/19	0/23	0/23	0/21	2/19

F: female; F_0: parental generation; F_1: first filial generation; M: male; N/A: not applicable; ppm: parts per million

Results presented as number of animals with loose stools / number of animals examined.

Source: Takahashi (1997)

440

Table 41. Incidence of caecum distension in three generations of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 ppm	1 200 ppm	6 000 ppm	30 000 ppm
F_0				
Males	0/24	0/24	0/24	21/24
Females	0/24	0/24	0/24	24/24
F_1				
Males	0/24	0/24	0/24	19/24
Females	0/24	0/24	0/24	17/24
Pups	0/136	0/141	0/143	89/141
F_2				
Pups	0/182	0/183	0/164	111/149

ppm: parts per million; F_0: parental generation; F_1: first filial generation; F_2: second filial generation

Results presented as number of animals with caecum distension / number of animals examined.

Source: Takahashi (1997)

No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment. Body weights of offspring at high doses were decreased in both generations, starting typically on postnatal day 14 (Table 42). Gross pathological examinations found an increased incidence of caecum distension in high-dose offspring of both generations.

Table 42. Mean body weights of two generations of offspring of rats administered glyphosate

	Mean body weights per dietary concentration of glyphosate							
	F_1 pups – male				F_2 pups – male			
PND	0 ppm	1 200 ppm	6 000 ppm	30 000 ppm	0 ppm	1 200 ppm	6 000 ppm	30 000 ppm
0	6.7 ± 0.6	6.8 ± 0.5	6.7 ± 0.4	7.2 ± 0.7*	7.0 ± 0.5	6.9 ± 0.6	7.3 ± 0.7	7.1 ± 0.5
4	11.6 ± 1.2	11.6 ± 1.2	11.7 ± 1.0	11.6 ± 1.2	12.0 ± 1.2	12.1 ± 1.5	12.5 ± 1.5	12.5 ± 1.3
7	19.5 ± 1.7	19.1 ± 2.0	19.5 ± 1.6	19.3 ± 1.2	19.8 ± 1.5	20.0 ± 1.9	20.4 ± 2.2	20.6 ± 1.7
14	39.5 ± 3.2	39.4 ± 2.6	39.3 ± 2.6	36.6 ± 2.6**	40.1 ± 3.0	39.0 ± 2.8	38.7 ± 2.9	39.1 ± 2.8
21	63.9 ± 4.4	63.8 ± 4.1	62.4 ± 3.7	55.1 ± 3.5***	58.6 ± 5.1	59.4 ± 4.4	58.3 ± 4.3	53.1 ± 4.4**
	F_1 pups – female				F_2 pups – female			
0	6.3 ± 0.6	6.4 ± 0.5	6.4 ± 0.5	6.8 ± 0.6*	6.6 ± 0.5	6.6 ± 0.7	6.8 ± 0.6	6.8 ± 0.6
4	11.1 ± 1.2	11.2 ± 1.1	11.3 ± 0.9	11.3 ± 1.2	11.6 ± 1.2	11.5 ± 1.6	12.0 ± 1.5	12.1 ± 1.1
7	18.6 ± 1.8	18.4 ± 1.9	18.8 ± 1.5	18.3 ± 1.6	18.9 ± 2.0	19.1 ± 2.1	19.6 ± 2.2	19.9 ± 1.4
14	38.4 ± 3.6	37.9 ± 2.6	38.2 ± 2.2	35.4 ± 2.6**	38.7 ± 3.5	38.0 ± 2.2	37.5 ± 2.9	38.1 ± 2.9
21	61.0 ± 4.8	60.6 ± 3.9	59.8 ± 3.1	53.2 ± 4.0***	56.4 ± 5.5	57.1 ± 4.4	56.2 ± 4.5	51.8 ± 4.2*

F_1: first filial generation; F_2: second filial generation; PND: postnatal day; ppm: parts per million; *: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$

Results presented are mean weights in grams ± standard deviation. Statistics from study report.

Source: Takahashi (1997)

The NOAEL for parental toxicity was 6000 ppm (equal to 417 mg/kg bw per day) based on increased incidence of loose stools and caecum distension in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day). As there were no effects on reproductive parameters the NOAEL for reproductive toxicity was 30 000 ppm (equal to 2150 mg/kg bw per day). The NOAEL for offspring toxicity was 6000 ppm (equal to 417 mg/kg bw per day) based on decreased pup body weights and increased incidence of caecum distension in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day) (Takahashi, 1997).

In a two-generation reproduction study, groups of 26 male and female Wistar-derived Alpk:APfSD rats (aged 5–6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity 97.6%) at concentrations of 0, 1000, 3000 or 10 000 ppm (equal to 0, 99.4, 292.6 and 984.7 mg/kg bw per day for males and 0, 104.4; 322.8 and 1054.3 mg/kg bw per day for females) for 10 weeks before their first mating and until termination. Each generation was mated twice avoiding sister/brother matings throughout. Males were terminated after completion of mating and females on or soon after day 29 of lactation, after which their organs were weighed and gross pathological and histopathological examinations conducted. The offspring not selected for mating were also terminated on day 29 postpartum, with one pup/sex per litter used for organ-weight determination and two pups /sex per litter given macroscopic examinations. All the remaining pups were terminated with no further examination.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed consumption, feed efficiency, organ weights or histopathological changes for parental animals of either generation. No adverse effects were observed for mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size and viability were not affected by treatment.

The body weights of F_{1A} pups were lower compared to the control group from day 8 onwards, but a similar effect was not seen in the F_{2A} pups. There was no treatment-related effect on total litter weight (Table 43).

Table 43. Mean body weights of two successive generations of offspring of rats administered glyphosate

	Mean body weights per dietary concentration of glyphosate (g)							
	Males				Females			
PND	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm
F_{1A}								
1	5.8	6.1	6.0	6.1	5.4	5.8	5.6	5.7
5	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1**
8	13.8	13.4	13.2	12.6*	13.3	12.8	12.4	12.1**
15	26.8	26.1	25.8	24.6*	26.1	25.2	24.5	23.8*
22	43.4	42.4	41.4	39.2*	41.9	40.3	39.4	37.7*
29	81.7	79.5	79.6	74.6*	77.1	74.0	74.1	69.9**
F_{2A}								
1	6.3	6.3	6.3	6.2	6.1	5.9	5.9	5.8
5	9.7	9.9	9.3	9.5	9.3	9.6	9.1	9.1
8	14.3	14.7	13.8	14.2	13.8	14.2	13.4	13.7
15	27.4	28.3	26.4	27.5	26.7	27.5	25.8	26.5
22	44.5	46.2	43.1	44.9	42.7	44.8	41.8	42.9

	Mean body weights per dietary concentration of glyphosate (g)							
	Males				Females			
PND	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm	0 ppm	1 000 ppm	3 000 ppm	10 000 ppm
29	83.0	86.0	80.6	82.8	77.7	80.6	75.6	77.4

F_{1A}: first filial generation, first litter; F_{2A}: second filial generation, second litter; PND: postnatal day; ppm: parts per million; *: $P = 0.05$ (Student t-test, 2 sided); **: $P = 0.01$ (Student t-test, 2 sided)

Source: Moxon (2000)

As no adverse effects were noted in the study, the NOAEL for parental and reproductive toxicity was 10 000 ppm (equal to 984.7 mg/kg bw per day), the highest dose tested. The NOAEL for offspring toxicity was 3000 ppm (equal to 292.6 mg/kg bw per day) based on reduced pup weights in the F_{1A} generation seen at 10 000 ppm; equal to 984.7 mg/kg bw per day (Moxon, 2000).

In a two-generation reproduction study, glyphosate (purity 95.7%) was administered in the diet to 28 Crl:CD(SD) IGS BR rats per sex per dose at 0, 1500, 5000 or 15 000 ppm (equal to 0, 104, 351 and 1063 mg/kg bw per day in males and 0, 126, 423 and 1273 mg/kg bw per day in females) for two successive generations with one litter per generation. After 10 weeks of treatment, animals were paired within each dose group on a 1:1 basis to produce the F_1 litters. At weaning, 24 males and 24 females from each dose group were selected to produce the F_2 generation. Surviving adult females and males and unselected offspring were terminated on day 21 postpartum. All adult animals and offspring underwent macroscopic examinations and parental organs were weighed. A small subset of organs were taken from one male and one female offspring from the F_0 and F_1 pairings (where available). Tissues from control and high-dose F_0 and F_1 animals underwent histopathological examination. As there were indications of changes in the adrenal glands of F_1 animals, microscopic examination was extended to include all dose groups.

No treatment-related adverse effects were observed on mortality, clinical signs, body weights, feed or feed efficiency, organ weights or histopathological changes in parental animals of either generation. No adverse effects were observed on mating performance, pregnancy rate or duration of pregnancy in either generation. Litter size, viability and offspring body weights were not affected by treatment. Complete preputial separation was delayed by 2.9 days in high-dose F_1 male pups (2.9 days) and body weights were increased by 10% at attainment. There were no treatment-related effects on the age or weight at attainment of vaginal opening.

As there were no effects for parental animals or on reproductive parameters, the NOAEL for parental and reproductive toxicity was 15 000 ppm (equal to 1063 mg/kg bw per day), the highest dose tested. The NOAEL for offspring was 5000 ppm (equal to 351 mg/kg bw per day), based on delayed age and increased weight at attainment of preputial separation at 15 000 ppm (equal to 1063 mg/kg bw per day) (Dhinsa, 2007).

(b) Developmental toxicity

Rats

In a pre-GLP developmental toxicity study, glyphosate (purity 98.7%) suspended in 0.5% aqueous Methocel was administered to 25 copulated CD female rats per dose by oral gavage at concentrations of 0, 300, 1000 or 3500 mg/kg bw per day from gestation day 6 through 19. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

Soft stools, diarrhoea, red nasal discharge, reduced activity and rales (abnormal respiratory noise) were noted in the highest dose group. By gestation day 17, six rats in this group had died. A

reduced mean body-weight gain due to a loss in mean maternal weight over the first three days of treatment was noted in the high-dose group. No significant differences between the 300 and 1000 mg/kg bw per day dosage groups and the control group were observed in terms of the mean number of viable fetuses, implantations, post-implantation losses, corpora lutea or mean fetal body weight. The mean number of total implantations, viable fetuses and mean fetal body weight were significantly decreased in the 3500 mg/kg bw per day dosage group compared to controls. In addition, the dams in the high-dose group had a significant increase in early resorptions, causing a slight increase in post-implantation losses.

At 3500 mg/kg bw per day, the number of litters with malformations was identical to that of the control group, but the number of fetuses with malformations was increased. However, since the number and type of malformations observed were similar to those observed in historical control data, it was concluded that they were not treatment related. There were an increased number of fetuses with unossified sternebrae in the high-dose group; although treatment related, this is considered a developmental variation rather than a teratogenic malformation. No malformations were observed in the 300 and 1000 mg/kg bw per day dosage groups.

The NOAEL for maternal toxicity was 1000 mg/kg bw per day based on mortality, soft stools and reduced body-weight gain at 3500 mg/kg bw per day. The NOAEL for developmental toxicity was 1000 mg/kg bw per day based on the decreased mean number of total implantations, viable fetuses, mean fetal body weight, increased early resorptions and increased number of fetuses with unossified sternebrae at 3500 mg/kg bw per day (Tasker, Rodwell & Jessup, 1980a).

In a developmental toxicity study, glyphosate (purity 98.6%) suspended in a 1.0% aqueous solution of methylcellulose was administered to 25 mated Crl:CD(SD)BR VAF/Plus female rats per dose by oral gavage at concentrations of 0, 300, 1000 or 3500 mg/kg bw per day from gestation days 6 through 15. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

At the highest dose, clinical abnormalities included salivation, loose stools and rales. The latter was also observed in two animals at the intermediate dose on one occasion. There were two maternal mortalities at the highest dose following signs of respiratory distress. Body-weight gain was markedly reduced at the highest dose (by 16–81% of control values, gestation days 6–20) and marginally reduced at the intermediate dose (by 86–97% of control values, gestation days 6–20). Feed consumption was slightly decreased at the highest dose during the dosing period (75–94% of control values, gestation days 6–15), but was comparable with controls thereafter. Water intake was increased at the highest dose (139–205% of control values, gestation days 6–15). No treatment-related changes were observed at any dose at necropsy.

A total of 23, 23, 25 and 22 dams had live young on day 20 in the control group and at 300, 1000 and 3500 mg/kg bw per day, respectively. Treatment had no significant effect on embryonic losses, litter size or sex ratio, but the litter weights were reduced at the highest dose (90% of control values) and mean fetal weights were statistically significantly reduced at the highest dose (94% of control values; $P < 0.01$). The occurrence of malformations was not significantly increased by treatment. However, the incidence of rib distortion (wavy ribs) was markedly higher at the highest dose and slightly higher at the intermediate dose; the incidences based on fetuses were 1, 0, 3 and 28 and on litters were 1, 0, 2 and 11 at 0, 300, 1000 and 3500 mg/kg bw per day, respectively. In addition, reduced ossification was seen slightly more frequently at the highest and intermediate doses. The percentage of fetuses showing skeletal anomalies (variations) was significantly increased at the two higher doses, but the percentage of fetuses affected at the intermediate dose exceeded the historical background range (21.9–27.2%) only slightly (Table 44).

444

Table 44. Skeletal anomalies in fetuses and litters of rats administered glyphosate

	Incidence per dietary concentration of glyphosate			
	0 mg/kg bw per day	300 mg/kg bw per day	1000 mg/kg bw per day	3500 mg/kg bw per day
Fetal anomolies[a]	19/155	36/143	46/166	55/142
Litter anomolies[b]	11/23	16/23	19/25	19/22
Fetal skeletal variations (%)[c]	11.7	22.6	28.4*	35.7**
Historical range	21.9–27.2			

bw: body weight; *: $P < 0.05$; **: $P < 0.01$

Kruskal–Wallis H-statistic followed, if significant, by intergroup comparison with control (distribution-free Williams' test).

[a] Results presented as number of fetuses with skeletal anomalies / total number of fetuses.

[b] Results presented as number of litters with skeletal anomalies / total number of litters.

[c] Results expressed as number of fetuses with skeletal variations (with malformed fetuses excluded) as a percentage of the total number of fetuses examined.

Source: Brooker et al. (1991a)

The NOAEL for maternal toxicity was 300 mg/kg per day based on clinical signs and reduced body-weight gain at 1000 mg/kg bw per day and higher. The NOAEL for developmental toxicity was 300 mg/kg per day based on an increased incidence of delayed ossification and an increased incidence of fetuses with skeletal anomalies at 1000 mg/kg bw per day and higher (Brooker et al., 1991a).

In a developmental toxicity study, glyphosate (purity 95.68%) suspended in a 0.5% aqueous solution of sodium carboxymethylcellulose was administered to 24 copulated Crj:CD(SD) female rats/dose by oral gavage at concentrations of 0, 30, 300 or 1000 mg/kg bw per day from gestation day 6 through 15. On gestation day 20, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in mortality, body weight, feed consumption or macroscopic findings in dams. An increased incidence of slightly loose stools was observed during the dosing period in 20 of the 22 pregnant females at 1000 mg/kg bw per day. Of these 20 animals, 9 still displayed the effect on the day after the last dosing.

There were no effects on number, growth or survival of fetuses. Any external, visceral or skeletal abnormalities were considered secondary to maternal toxicity; furthermore, the effects were also seen in the control group, incidences of the effects were low and/or there was no dose–response relationship for the effect.

The NOAEL for maternal toxicity was 300 mg/kg bw per day based on the increased incidence of slightly loose stools observed in dams at 1000 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 1000 mg/kg bw per day (Hatakenaka, 1995).

In a developmental toxicity study, glyphosate acid (purity 95.6%) in deionized water was administered to 24 time-mated female Alpk:APfSD (Wistar-derived) rats/dose by oral gavage at 0, 250, 500 or 1000 mg/kg bw per day from gestation day 7 through 16. On gestation day 22, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All the fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

One control animal was terminated on day 7 due to incorrect dosing. There were no treatment-related changes in clinical observations, body weight, feed consumption or macroscopic findings for dams.

There were no effects on number, growth or survival of fetuses and no treatment-related external, visceral or skeletal abnormalities.

As there were no maternal or developmental effects, the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day (Moxon, 1996a).

Rabbits

In a developmental toxicity study, glyphosate (purity 98.7%) suspended in a 0.5% aqueous Methocel solution was administered to 16 Dutch Belted female rabbits per dose by oral gavage at concentrations of 0, 75, 175 or 350 mg/kg bw per day from gestation day 6 through 27. On gestation day 28, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities. This study was conducted prior to GLP.

Incidence of mortality was increased in the high-dose group. The number of spontaneous deaths in the control, low-, mid- and high-dose groups was 0/16, 1/16, 2/16 and 10/17, respectively. A slight increase in the incidence of soft stools and diarrhoea was noted in the medium–high-dose group (individual data not reported). At 350 mg/kg bw per day, soft stool and/or diarrhoea were observed in each animal at least once during treatment. An increased incidence of nasal discharge was also noted in the high-dose group (individual data not reported). There were no treatment-related changes in body weight or macroscopic findings for dams.

Due to the increased mortality at the high dose, the number of animals (6 pregnant females) available for evaluation of developmental effects was insufficient. The numbers of pregnant dams were also low for the other doses (12, 15 and 11 in the control, low- and mid-dose groups, respectively), limiting the evaluation of developmental effects in this study. The available data for the control, low- and mid-dose groups indicate no treatment-related adverse effects on the number, growth or survival of fetuses. Any external, visceral or skeletal abnormalities were not considered treatment related.

The NOAEL for maternal toxicity was 175 mg/kg bw per day based on increased incidence of clinical signs (soft stools and diarrhoea) and mortality at 350 mg/kg bw per day in rabbits. Individual data were not provided for the clinical signs at 175 mg/kg bw per day, and the increase in incidence was only slight at this dose. Due to the low number of pregnant dams, developmental effects could not be evaluated; however, the available data indicate no evidence of developmental effects (Tasker, Rodwell & Jessup, 1980b).

In a developmental toxicity study, glyphosate (purity 95%) suspended in a 0.1% aqueous gum acacia solution was administered to 15 New Zealand White female rabbits per dose by oral gavage at concentrations of 0, 125, 250 and 500 mg/kg bw per day, respectively, from gestation day 6 through 18. On gestation day 29, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in mortality, feed consumption or macroscopic findings for dams. Two abortions were noted in the high-dose group. A slight decrease in body-weight gain was also noted at 500 mg/kg bw per day.

There were no treatment-related adverse effects on the number, growth or survival of fetuses. The mean number of viable implants per litter was lower at the high dose than the other treatment groups and controls and accordingly the mean number of non-viable implants per litter was higher

than the other treatments groups; however, when taking into account the variability for these measurements, the changes were not considered adverse.

Incidences of external, visceral or skeletal variations/malformations in fetuses in the low- and mid-dose groups did not differ from those of the control group (Table 45). At 500 mg/kg bw per day, incidences of variations/malformations were higher than in the control group, but in many cases the increase was minimal or similar to the 125 and 250 mg/kg bw per day dose groups when evaluated on a litter basis. These increases in incidences of variations/malformation were observed in the presence of severe maternal toxicity. The occurrences of a variety of low-incidence fetal effects (malformations) were slightly increased at higher dose levels. These increases are considered secondary to maternal toxicity.

Table 45. Malformations and variations in fetuses and litters of rabbit administered glyphosate

	Incidence per dietary concentration of glyphosate			
Malformations / variations	**0 mg/kg bw per day**	**125 mg/kg bw per day**	**250 mg/kg bw per day**	**500 mg/kg bw per day**
Number of litters examined	13	14	14	12
Number of fetuses examined	109	113	120	78
Malformations				
Tail abnormal	1 (1)	1 (1)	2 (2)	3 (2)
Low-set ears	0 (0)	1 (1)	1 (1)	2 (1)
Ventricular septal defect	0 (0)	1 (1)	1 (1)	2 (2)
Postcaval lung lobe absent	0 (0)	1 (1)	2 (2)	4 (3)
Kidney(s) absent	1 (1)	2 (2)	2 (2)	6 (4)
Rudimentary rib (no. 14)	1 (1)	0 (0)	2 (2)	5 (2)
Variations				
Tail blunt tipped	1 (1)	0 (0)	3 (2)	5 (4)
Irregular rugae on palate	0 (0)	2 (1)	3 (2)	2 (2)
Lateral ventricles of cerebrum dilated	0 (0)	2 (2)	2 (2)	6 (4)
Right ventricle smaller than normal	1 (1)	3 (2)	3 (2)	5 (3)
Globular heart	2 (2)	0 (0)	3 (2)	5 (4)
Incomplete separation of lung lobes	1 (1)	2 (1)	2 (1)	4 (2)
Parietal fetal atelectasis	0 (0)	1 (1)	1 (1)	1 (1)
Liver irregular shape	0 (0)	2 (1)	2 (2)	6 (4)
Kidney(s) globular shape	0 (0)	0 (0)	2 (1)	5 (3)
Cervical central 1–3 and/or 4 bilobed	1 (1)	0 (0)	1 (1)	2 (2)
Anterior arch of the atlas poorly ossified	2 (1)	2 (1)	1 (1)	4 (2)
Anterior arch of the atlas split	0 (0)	0 (0)	2 (1)	3 (1)
Extrathoracic centrum and arch	1 (1)	3 (2)	2 (1)	5 (3)
Thoracic centrum only one ossification centre	1 (1)	0 (0)	1 (1)	3 (2)
Thoracic centra fused	2 (1)	1 (1)	1 (1)	2 (1)
Extra ribs on thoracic centra and arch 13 bilateral	1 (1)	0 (0)	3 (2)	5 (4)
Sternebra – 6 poorly ossified	2 (1)	1 (1)	2 (1)	4 (2)
Sternebra(e) split	2 (1)	2 (1)	1 (1)	5 (3)
Sternebra(e) unossified	3 (2)	1 (1)	3 (2)	6 (4)

449

Malformations / variations	Incidence per dietary concentration of glyphosate			
	0 mg/kg bw per day	125 mg/kg bw per day	250 mg/kg bw per day	500 mg/kg bw per day
Number of litters examined	13	14	14	12
Number of fetuses examined	109	113	120	78
Pubis, poorly ossified	3 (2)	2 (2)	3 (1)	4 (3)
Some ossification in knee area	1 (1)	3 (2)	2 (1)	2 (2)
Skull bones poorly ossified	1 (1)	3 (2)	2 (1)	2 (2)
Frontal, hole in bone	0 (0)	1 (1)	2 (2)	2 (2)
Reduced number of caudal segments	1 (1)	2 (2)	1 (1)	3 (2)

bw: body weight

Results presented as number of fetuses with malformations and variations and, in parentheses, the number of litters with malformations and variations.

Source: Bhide & Patil (1989)

The NOAEL for maternal toxicity was 250 mg/kg bw per day based on abortions observed at 500 mg/kg bw per day in rabbits. The NOAEL for developmental toxicity was 250 mg/kg bw per day based on increased incidence of variations/malformations observed at 500 mg/kg bw per day in rabbits. It should be noted that individual data, uterine weights, maternal necropsy results and statistical analyses were not provided for this study; therefore, the NOAEL and LOAEL values are based on the available data (Bhide & Patil, 1989).

In a developmental toxicity study, glyphosate acid (purity 98.6%) suspended in a 1% aqueous methylcellulose solution was administered to 19, 19, 16 or 20 New Zealand White rabbits per dose by oral gavage at concentrations of 0, 50, 150 or 450 mg/kg bw per day, respectively, from gestation day 7 through 19. On gestation day 29, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in body weight, feed consumption or macroscopic findings for dams. One high-dose animal was found dead on day 20 following signs of abortion on day 19 and soft/liquid faeces, a reduction in feed intake and body-weight loss from the start of treatment. The incidence of soft/liquid faeces was increased at the high dose (13/20 animals).

There were no treatment-related adverse effects on the number, growth or survival of fetuses. At termination, 18, 12, 15 and 13 pregnant females were available for evaluation in the control, low, mid and high doses, so evaluation of developmental effects is limited at the low and high doses. Embryo/fetal death and post-implantation loss were increased in all treatment groups; however, there was no dose–response and the values were within or slightly above the historical control range.

Any external, visceral or skeletal abnormalities were not considered treatment related. There was a slightly increased incidence of cardiac malformation (interventricular septal defect) at the high dose (4/13 pregnant animals); however, it was barely outside of the historical control range from studies conducted during the same period, and the number of litters to evaluate this dose was reduced. Furthermore, this effect was considered secondary to the maternal toxicity observed at 450 mg/kg bw per day.

The NOAEL for maternal toxicity was 150 mg/kg bw per day based on clinical signs (soft/liquid faeces) at 450 mg/kg bw per day in rabbits. The NOAEL for developmental toxicity was 150 mg/kg bw per day based on the post-implantation loss, late embryonic death and an increase in cardiac malformations at 450 mg/kg bw per day (Brooker et al., 1991b).

In a developmental toxicity study, glyphosate acid (purity 96.8%) suspended in a 0.5% aqueous CMC solution was administered to 26, 17, 16 and 16 presumed-mated New Zealand White rabbits per dose by oral gavage at concentrations of 0, 20, 100 or 500 mg/kg bw per day, respectively, from gestation day 6 through 18. On gestation day 28, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All the fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in body weight, feed consumption or macroscopic findings for dams. There were two, zero, four and eight deaths in the control, low-, mid- and high-dose groups, respectively; the deaths in the control group were definitively attributed to gavage error. An increased incidence of soft stool/liquid faeces was observed at the high dose (12/15 animals). Other clinical signs at the high dose included rales, weakness, dyspnoea and ocular discharge; however, the incidence of these effects was low and some effects may indicate gavage error. At necropsy, various findings were noted in the lungs and trachea in mid- and high-dose animals, which also suggests possible gavage errors and/or issues with animal husbandry.

There were no treatment-related adverse effects on the number, growth or survival of fetuses. However, the number of pregnant females available for evaluation in the control and the low-, mid- and high-dose groups was 20, 13, 12 and 6, respectively, limiting the study of developmental effects. Total litter loss was recorded for one female in the high-dose group. Any external, visceral or skeletal abnormalities were not considered treatment related. Major visceral malformations primarily affected the heart, but occurred in single incidences and/or showed no dose–response relationship except for the dilated heart; however, interpreting the dose–response relationship is difficult given the limited number of litters available, especially at the high dose. In addition, this effect was considered secondary to the maternal toxicity observed at 500 mg/kg bw per day.

Based on the uncertainties regarding gavage errors and mortalities across doses in this study and the reduced number of pregnant females, the study is considered unacceptable (Suresh, 1993c).

In a developmental toxicity study, glyphosate (purity 97.56%) suspended in a 0.5% aqueous solution of sodium carboxymethylcellulose was administered to 18 artificially inseminated Japanese white rabbits (Kbl:JW) per dose by oral gavage at concentrations of 0, 10, 100 or 300 mg/kg bw per day from gestation day 6 through 18. On gestation day 27, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in body weight, feed consumption or macroscopic findings for dams. One dam died on gestation 20 without showing any clinical signs, and the cause of death was undetermined. An increased incidence of loose stools was observed during the dosing period in four of the 17 remaining pregnant females In the high-dose group; two continued to display this effect during the post-dosing period and one aborted on gestation day 26.

There were no effects on number, growth or survival of fetuses. All observations of external or visceral malformations were sporadic in nature and not considered treatment related. Skeletal malformations and variations were also not considered treatment-related since these effects were also seen in the control group, incidences of the effects were low and/or there was no dose–response relationship for the effect.

The NOAEL for maternal toxicity was 100 mg/kg bw per day based on the increased incidence of loose stools observed in dams at 300 mg/kg bw per day. There were no developmental effects; therefore, the NOAEL for developmental toxicity is 300 mg/kg bw per day (Hojo, 1995).

In a developmental toxicity study, glyphosate (purity 95.3%) suspended in a 1% CMC was administered to 18 mated New Zealand White female rabbits per dose by oral gavage at concentrations of 0, 50, 200 or 400 mg/kg bw per day from gestation day 7 through 19. On gestation day 29, the dams were terminated, pregnancy status determined and numbers of corpora lutea,

implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related changes in body weight, feed consumption or macroscopic findings for dams. One high-dose female was found dead prior to dosing on day 19 and another was terminated in extremis on day 20; one death also occurred in the control group and in the mid-dose group. An increased incidence of diarrhoea was observed at the high dose in 10 of the 16 surviving pregnant females. All other clinical observations were isolated or a dose–response relationship was not observed.

There were no treatment-related adverse effects on the number, growth or survival of fetuses. The increases in late fetal deaths and post-implantation loss noted at the high doses were not considered adverse once the variability in the measurements were taken into consideration. In addition, the increase can mainly be attributed to one animal with nine late-death fetuses. No treatment-related external, visceral or skeletal abnormalities were observed.

The NOAEL for maternal toxicity was 200 mg/kg bw per day based on increased incidence of diarrhoea in dams at 400 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 400 mg/kg bw per day (Coles & Doleman, 1996).

In a developmental toxicity study, glyphosate acid (purity 95.6%) in deionized water was given to 20 time-mated New Zealand White female rabbits per dose by oral gavage at concentrations of 0, 100, 175 or 300 mg/kg bw per day from gestation day 8 through 20. On gestation day 30, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no treatment-related adverse changes in mortality, body weight, feed consumption or macroscopic findings for dams. There was a significant increase in the incidence of either diarrhoea or decreased faecal output at the mid and high doses (no statistical significance was provided) (Table 46). The incidence of staining in the genital area was also increased at the high dose.

Table 46. Clinical signs in pregnant rabbits administered glyphosate by gavage

		No. per dietary concentration of glyphosate		
Clinical sign	**0 mg/kg bw per day**	**100 mg/kg bw per day**	**175 mg/kg bw per day**	**300 mg/kg bw per day**
Few faeces in tray	3	3	9	9
Signs of diarrhoea	4	5	11	19
Staining in genital area	2	2	3	11

bw: body weight; no. number

Source: Moxon (1996b)

There were no treatment-related adverse effects on the number, growth or survival of fetuses. Although mean fetal weight was reduced at the high dose, this was not considered adverse once the variability in the measurements was taken into account. In addition, the decrease could be attributed to two litters with lower weights. Any external, visceral or skeletal abnormalities were not considered treatment related.

The NOAEL for maternal toxicity was 100 mg/kg bw per day based on increased incidence of clinical signs (decreased faecal output or signs of diarrhoea) in rabbits at 175 mg/kg bw per day. As there were no developmental effects, the NOAEL for developmental toxicity was 300 mg/kg bw per day (Moxon, 1996b).

450

205

2.6 Special studies

(a) Neurotoxicity

Cell cultures

In a non-guideline experiment, a cell culture model was used to determine if chronic exposure to organophosphate pesticides can alter the sensitivity of nerve cells to subsequent acute exposure to organophosphates or other compounds. NB2a neuroblastoma cells were grown in the presence of diazinon at a concentration of 25µmol/L for 8 weeks. The organophosphate was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides, including glyphosate (purity > 99%). The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the median inhibitory concentration (IC_{50}) for each organophosphate or formulation calculated. The IC_{50} values in cells pre-exposed to diazinon were compared with the equivalent values in cells not pre-exposed to diazinon. The IC_{50} for inhibition of neurite outgrowth by acute application of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20% and 90% after pretreatment with diazinon.

According to the study authors, the data support the view that long-term exposure to an organophosphate may reduce the threshold for toxicity of some environmental agents (Axelrad, Howard & McLean, 2003).

Rats

In an acute neurotoxicity study, groups of fasted (24 hours), approximately 42-day-old Alpk:APfSD rats (10/sex per dose) were given a single oral dose of glyphosate (purity 95.6%) in deionized water at concentrations of 0, 500, 1000 or 2000 mg/kg bw. They were then observed for 2 weeks. Neurobehavioural assessment (functional observational battery and motor activity testing) was performed in all animals in week −1 (pre-dosing), on day 1 (approximately 6 hours after dosing), day 8 and day 15. At study termination, five animals/sex per dose were euthanized and perfused. Of the perfused animals, the control and highest dose groups were used for neuropathological examinations with brain and peripheral nervous system tissues undergoing histopathological evaluation.

Administration of a single dose of glyphosate produced treatment-related clinical signs of general toxicity at 2000 mg/kg bw. On day 1, approximately 6 hours after dosing, three high-dose females were observed with decreased activity, subdued behaviour, hunched posture and/or hypothermia. Diarrhoea was also seen in another female at this dose. Full recovery was established by day 2. These clinical signs do not reflect signs of neurotoxicity and were mostly likely associated with the excessively high dose of glyphosate. No treatment-related effects were observed on mortality, body weight or brain weight. Similarly, neuropathological and histopathological examinations showed no treatment-related effects, and functional observational battery and motor activity tests revealed no treatment-related effects. Although overall motor activity at 2000 mg/kg bw for both sexes on day 1 was lower than that of controls, these differences were not statistically significant or dose dependent.

The NOAEL for neurotoxicity in rats was 2000 mg/kg bw. The NOAEL for systemic toxicity was 1000 mg/kg bw based on clinical signs of general toxicity (decreased activity, subdued behaviour, hunched posture, hypothermia and diarrhoea) and lethality at 2000 mg/kg bw. The LOAEL for systemic toxicity in rats was 1000 mg/kg bw (Horner, 1996a).

In a subacute neurotoxicity study, glyphosate (purity 95.6%) was administered to 12 Alpk:APfSD rats per sex per group in the diet at concentrations of 0, 2000, 8000 or 20 000 ppm (equal to 0, 155.5, 617.1 and 1546.5 mg/kg bw per day for males and 0, 166.3, 672.1 and 1630.6 mg/kg bw per day for females) for 13 weeks. Neurobehavioural assessment (functional observational battery and motor activity testing) was performed in all animals at weeks −1, 1, 5, 9 and 14. At study termination, six animals/sex per group were euthanized and perfused. Of these, the control and highest

dose groups were used for neuropathological examinations and brain and peripheral nervous system tissues histopathologically evaluated.

Overall mean body weight (92.8% of the controls; $P < 0.05$) and feed utilization ($P < 0.01$) were reduced in high-dose males with no treatment-related effect on feed consumption. Group mean body-weight was also lower than the controls in males at 8000 ppm from weeks 6–14 (not statistically significantly). No treatment-related effects on mortality, clinical signs or brain weight were observed. Functional observational battery and locomotor activity testing revealed no treatment-related effects. Neuropathological and histopathological examinations of the peripheral and nervous system did not yield any treatment-related effects from glyphosate administration.

The NOAEL for neurotoxicity in rats was 20 000 ppm, equal to 1547 mg/kg bw per day. The NOAEL for systemic toxicity was 20 000 ppm, equal to 1546.5 mg/kg bw per day (Horner, 1996b).

Hens

In an acute delayed neurotoxicity study, 20 hens (hybrid brown laying strain – Lohmann Brown) were given a single oral dose of glyphosate (purity 95.6%) of 2000 mg/kg bw. In addition, 12 negative control hens were dosed with distilled water and 12 positive control hens with 1000 mg/kg bw of triorthocresyl phosphate (TOCP). This was followed by an observation period of 21/22 days. The hens were examined for any clinical signs twice daily and for ataxia daily, and weighed weekly. Brain acetylcholinesterase, brain neuropathy target esterase (NTE) and lumbar spine NTE measurements were made on three hens, 48 hours after dosing. At the end of the observation period, six hens from each treatment group were selected for termination and macroscopic and histopathological examination. After perfusion through the heart with fixative, the selected tissues were processed and examined histopathologically.

No treatment-related mortality was observed in the study. There was no evidence of clinical ataxia in any of the negative controls or in any of the hens dosed with glyphosate. Of the 12 hens dosed with TOCP (positive controls), five developed clinical ataxia, starting between days 11 and 21. There was no effect on body weights for hens dosed with glyphosate, but TOCP-dosed hens showed an overall weight loss. Acetylcholinesterase was reduced by 6% in glyphosate-treated hens and 19% in TOCP-treated hens. There was no effect on NTE levels in brain or spinal cord for the glyphosate-treated hens, but compared to the negative controls, brain NTE levels were reduced by 84% and spinal cord NTE levels by 78% in the positive controls. No macroscopic abnormalities were seen in any of the hens examined. Histopathological examination revealed no evidence of acute delayed neurotoxicity or any other treatment-related changes in glyphosate-treated hens. Hens dosed with TOCP showed significant axonal degeneration in spinal cord, peripheral nerve and cerebellum, demonstrating the validity of the test system.

In conclusion, oral administration of a single dose of 2000 mg/kg bw of glyphosate produced no clinical signs of delayed neurotoxicity, no significant reduction in acetyl cholinesterase and no histopathological findings in hens. The NOAEL for acute delayed neurotoxicity of glyphosate in hens was 2000 mg/kg bw (Johnson, 1996).

(b) Immunotoxicity

In an unpublished immunotoxicity study, glyphosate (purity 85.2%) was administered to female B6C3F1/Crl mice (10/dose) in the diet at dose levels of 0, 500, 1500 or 5000 ppm (equal to 0, 150.1, 449.1 and 1447.5 mg/kg bw per day, respectively) for 28 days. The positive control group (10 females) was administered 50 mg/kg bw per day of cyclophosphamide (10 mL/kg at a concentration of 5 mg/mL) by intraperitoneal injection from study days 24–27. On day 24, all the animals in all the groups received a single intravenous dose of 7.5 × 107 sheep red blood cells (SRBC) in 0.2 mL of Earle's Balanced Salt Solution with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid. At termination, the spleen and thymus were removed and weighed. The T-cell–dependent antibody response to SRBC was measured with antibody-forming cell (AFC) assay.

452

There were no pre-terminal deaths, no treatment-related clinical signs and no treatment-related effects on feed and water consumption, mean body weights, organ weights and macroscopic findings in all treated groups. The body weights of the positive control group treated with cyclophosphamide did not differ significantly from those of the vehicle control group, but the absolute and relative spleen and thymus weights decreased statistically significantly ($P < 0.01$).

The systemic NOAEL was 5000 ppm (equal to 1448 mg/kg bw per day), the highest dose tested.

No statistically significant differences were observed in anti-SRBC antibody-forming cell responses for specific activity (AFC/106 spleen cells) and total spleen activity (AFC/spleen) in treated groups compared to the vehicle control group. The positive control group had a statistically significant ($P < 0.05$) decrease in spleen cell numbers, mean specific activity and mean total spleen activity. This confirmed the ability of the test system to detect immunosuppressive effects and confirmed the validity of the study design. Natural killer cell activity was not evaluated in this study.

The NOAEL for immunotoxicity was 5000 ppm (equal to 1448 mg/kg bw per day), the highest dose tested (Haas, 2012).

In a published study, female CD-1 mice were exposed to Tordon 202C (2,4-dichlorophenoxyacetic acid [2,4-D] and picloram) or Roundup in drinking water for 26 days at concentrations from 0–0.42% or 0–1.05%, respectively. Glyphosate isopropylammonium salt was administered in distilled drinking water at concentrations of 0%, 0.35%, 0.70% or 1.05% (approximately equal to 335, 670 and 1000 mg/kg bw). The mice were inoculated with SRBC to produce a T-lymphocyte macrophage-dependent antibody response on day 21 of the herbicide exposure period. Roundup exposure did not alter weight gain or water consumption. Antibody production was also unaffected by Roundup dosing, suggesting that Roundup is unlikely to cause immune dysfunction under normal conditions of application (Blakley, 1997).

The role of glyphosate in developing asthma and rhinitis among farmers was evaluated in a published study. The aim of this study was to explore the mechanisms of glyphosate-induced pulmonary pathology by utilizing murine models and real environmental samples. C57BL/6, TLR4_/_, and IL-13_/_ mice inhaled extracts of glyphosate-rich air samples collected on farms during spraying of herbicides or inhaled different doses of glyphosate and ovalbumin. The cellular response, humoral response and lung function of exposed mice were evaluated. Inhalation exposure to glyphosate-rich air samples as well as glyphosate alone increased eosinophil and neutrophil counts, mast cell degranulation and production of the cytokines interleukin-33 (IL-33), thymic stromal lymphopoietin, interleukin-13 (IL-13) and interleukin-5 (IL-5). In contrast, in vivo systemic interleukin-4 (IL-4) production was not increased. Co-administration of ovalbumin with glyphosate did not substantially change the inflammatory immune response. However, deficiency in IL-13 resulted in diminished inflammatory response, but did not have a significant effect on airway resistance upon methacholine challenge after 7 or 21 days of glyphosate exposure. Glyphosate-rich farm air samples as well as glyphosate alone were found to induce pulmonary IL-13–dependent inflammation and promote Th2-type cytokines, but not IL-4 for glyphosate alone (Kumar et al., 2014).

(c) Effects on the salivary gland

Groups of 24 male Alpk:AP$_f$SD (Wistar-derived; AP), Sprague Dawley (Charles River; CD) and Fischer 344 (F344) rats were fed diets containing 0 or 20 000 ppm glyphosate acid for 28 consecutive days. Eight animals from each group were terminated of day 29, and the remaining rats retained without treatment for an additional 4 (eight rats/group) or 13 weeks (eight rats/group).

Dietary exposure to 20 000 ppm glyphosate acid resulted in significant reductions in body weight and minor reductions in feed consumption in AP and CD rats, but not in F344 rats. Salivary gland weight was unaffected in the CD rat but was increased in both AP and F344 rats at the end of the 4-week dietary exposure period. Microscopic examination of the salivary glands showed that the most pronounced effected occurred in the F344 strain, where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells occurred in the AP and CD strains.

After four weeks on the control diet, the salivary glands of the F344 strain had significantly recovered, while AP and CD rats were indistinguishable from their corresponding controls.

After 13 weeks on the control diet, slightly more glyphosate-treated F344 rats showed minor focal changes in the salivary glands compared to their controls, and group mean salivary-gland weights were increased slightly (Allen, 1996).

In a study of the mechanism of induction of salivary gland lesions performed by the National Toxicology Program, two groups of four male F344/N rats were fed diets containing glyphosate (purity 99%) at a concentration of 50 000 ppm (the highest dose used in a short-term study on toxicity) and given a continuous subcutaneous infusion of propanolol (a β-blocker; 1.2 mg/kg bw per day) or a vehicle (water). Three additional groups of four male rats were fed a control diet and given a continuous subcutaneous infusion of isoproterenol (a β-adrenergic agonist; 1.0 mg/kg bw per day), isoproterenol plus propanolol, or a vehicle (water). After 14 days of treatment, the animals were terminated, and the parotid and submandibular/sublingual glands were removed, weighed and processed for electron and light microscopy.

All the rats survived to the end of the study. Rats subcutaneously infused with isoproterenol were hypoactive and had increased respiratory rates on day 1, but behaved normally by the following day. While there was no effect on feed consumption in any group, there was a significant decrease in body-weight gains in the groups fed glyphosate (6.3 g and 6.0 g, compared with 16.0 g in controls). Both glyphosate and isoproterenol produced increased salivary-gland weights, with the parotid gland being more affected (280% or 154% of weights in the control group for glyphosate or isoproterenol, respectively). When both compounds were given along with propanolol, parotid weights were 194% of those of the controls for glyphosate but only 109% of those of the controls for isoproterenol. In the parotid and in the submandibular gland, increased weights were associated with cytoplasmic changes of acinar cells (basophilic change, fine vacuolation, swelling, loss of the normal positive periodic acid–Schiff reactivity of the secretory granules). The study authors concluded that the salivary gland effects induced by glyphosate were mediated through an adrenergic mechanism (Chan & Mahler, 1992).

The hypothesis that glyphosate produced the changes to the salivary gland via β-adrenergic activity was questioned in a recent review paper (Williams, Kroes & Munro, 2000). The authors emphasized that if glyphosate was a β-agonist, it would stimulate β-receptors in other effector organs and produce a characteristic set of cardiocirculatory effects, such as increased heart rate and cardiac output as well as decreased blood pressure and peripheral resistance; none of these effects were noted in other toxicological studies. Similarly, it is known that isoproterenol and other β-agonists cause myocardial necrosis and enlargement of heart ventricles after prolonged treatment. Glyphosate did not produce any effects in heart tissue, even after long-term exposure at very high doses, further supporting for the argument that glyphosate does not act as a β-agonist. The authors concluded that glyphosate has no significant β-adrenergic activity and could not produce salivary-gland changes via β-agonist activity. They proposed a number of other potential mechanisms for salivary gland alteration, including non-chemical modes of action. For example, salivary gland secretion has been shown to be affected by the texture and moistness of feed, and salivary gland enlargement has been caused by malnutrition. Glyphosate could be acting by just such a non-chemical mechanism. Because glyphosate is a strong organic acid, dietary administration at relatively high concentrations may cause

mild oral irritation leading to increased salivary gland size and flow. In the long-term exposure studies with glyphosate, there were several changes to the salivary glands. These changes were most pronounced in the parotid gland, responsible for secreting serous fluid in response to stimuli such as acidic materials; absent in the sublingual gland that releases mucous fluid in response to other stimuli; and observed to an intermediate degree in the submandibular gland that contains a mixture of mucous and serous secreting cells. This pattern of observations was considered consistent with the hypothesis that the changes are a biological response to the acidic nature of glyphosate. These alterations are not known to represent any pathological condition and were not considered toxicologically significant or adverse (Williams, Kroes & Munro, 2000).

A 2-month exploratory study evaluated the effects of a low pH diet on the parotid salivary glands of rats. Five groups, each with 10 male Crl:CD (SD) rats, were dosed for 56 consecutive days. Group 4 animals were fed a low pH diet containing 14 000 ppm citric acid, and group 5 a high pH diet with 21 400 ppm trisodium citrate dihydrate and a citrate ion concentrate equivalent to group 4's. Group 2, the controls, were fed the basal diet. Group 3 were administered citric acid in deionized water by gavage at 791–1316 mg/kg bw per day, with the dosing calculated to maintain citric acid dose levels approximately equal to group 4's. Group 1 were gavaged with deionized water.

Treatment-related effects consisted of statistically significant higher parotid salivary-gland weights in group 4, compared to the group 2 controls. The higher parotid salivary-gland weights seen in groups 3 (gavaged citric acid) and 5 (fed a trisodium citrate dihydrate diet) were not statistically significant.

The report states that with the absence of microscopic findings such as cytotoxicity and hyperplasia, the observed effects are likely adaptive responses to the low pH diet causing local irritation in the oral cavity rather than adverse effects (Haas, 2010).

A 4-month study examined the effects of glyphosate acid on the salivary glands of different rat strains (AP, CD and F344) after feeding diets containing 20 000 ppm glyphosate acid to male rats for 28 consecutive days and monitoring recovery over 4 or 13 weeks.

Differences in terms of systemic toxicity (changes in body weight and feed consumption) were minor, but marked differences were seen in the severity of effect on the parotid salivary gland. Significant reductions in body weight with minor reductions in feed consumption were seen in AP and CD rats but not in F344 rats. In contrast, salivary-gland weight was unaffected in CD rats but was increased in both AP and F344 rats. Microscopic examination showed the most pronounced effect to be in F344 rats where cytoplasmic basophilia were diffuse and parotid acinar cells enlarged. Similar but lesser effects involving only small foci of cells occurred in the AP and CD strains.

Complete recovery was seen in AP and CD rats following the 4-week recovery period. Although significant recovery of salivary-gland change was observed in F344 rats, it may not have been complete after a 13-week recovery period (Wood, 1996).

In an in vivo study, five male and five female Sprague Dawley (CD) rats were dosed with glyphosate technical (purity 95.3%) at a dose level of 5000 mg/kg bw with similar sized control groups receiving the vehicle only. Approximately 1 hour after dosing, control and treated animals were examined for either haematological, electrocardiographic or behavioural/functional changes. There were no differences in response between treated and control animals.

Ex vivo studies evaluated the effect of saturated solutions of glyphosate technical on isolated guinea pig ileum and isolated rat gastrocnemius muscle. Glyphosate technical caused a contractile response in isolated guinea pig ileum similar to that seen with acetylcholine; the effect was negated when the ileum was pre-incubated with atropine sulfate (Wood, 1996).

(d) Gastrointestinal tract irritation

In a study comparing the irritant effects on the stomach and ileum of a glyphosate formulation containing isopropylamine salt (41%) and surfactant (15%) with hydrochloric acid, a Teflon-coated catheter was inserted into intestinal duct of beagle dogs to administer each ration of the test solutions. Each sample was left in the stomach and intestine for 30 minutes and then the tissues were washed with physiological saline and examined. Based on the histopathological findings, the study concluded that the mucosal damage in the stomach and intestine caused by glyphosate formulation was mild, equivalent to that caused by 0.25 eq/L hydrochloric acid. The intestine was more severely damaged than the stomach in every case (Mizuyama, 1987).

(e) Endocrine disruption

For the USA pesticide regulatory risk assessment, the USEPA Endocrine Disruptor Screening Program (EDSP) Tier 1 assay battery is designed to provide the necessary empirical data to evaluate the potential of chemicals to interact with the estrogen-, androgen- or thyroid-signalling pathways. This interaction includes agonism and antagonism at estrogen and androgen receptors as well as at the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–thyroid axes, and altered steroidogenesis. In determining whether glyphosate interacts with estrogen-, androgen- or thyroid-signalling hormone pathways, the number and type of effects induced, the magnitude of responses and the pattern of responses observed across studies, taxa and sexes were considered. In addition, the conditions under which effects occur were considered, and in particular, whether endocrine-related responses occurred at doses that also resulted in systemic or overt toxicity.

This evaluation re-examines the data evaluated by the EDSP Tier 1 Assay Weight-of-Evidence Review Committee of the Office of Pesticide Programs as well as the Office of Science Coordination and Policy weight-of-evidence analysis of the potential interaction of glyphosate with the estrogen, androgen or thyroid hormone pathways, conducted on September 17, 2014, and concurs with the overarching conclusions.

For the estrogen pathway, there was no evidence of potential interaction of glyphosate with the estrogen pathway in the EDSP Tier 1 in vitro assays (i.e. estrogen-receptor binding assay, estrogen-receptor transactivation assay, aromatase and steroidogenesis assays). While glyphosate has been reported to show estrogen-receptor agonism in vitro with estrogen-dependent human breast cancer cells (Thongprakaisang et al., 2013), there were confounding issues with this study, and other in vitro estrogen receptor studies with glyphosate have not demonstrated an interaction (e.g. Kojima et al., 2004).

In addition, glyphosate was negative in the Tier 1 in vivo mammalian assays (i.e. uterotrophic or female pubertal assays). In the fish short-term reproduction assay (FSTRA), the non-treatment-responsive decrease (only significant at mid-treatment) in vitellogenin (VTG) was seen in isolation in the absence of any treatment-related effects in the other estrogen-related end-points such as gonado-somatic index, gonadal staging, fecundity and fertilization. In addition, there was no notable gonadal histopathology. In the open literature, glyphosate did not increase plasma VTG in juvenile rainbow trout (Xie et al., 2005). There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 US Toxicological Data Requirement mammalian or wildlife studies (only decreases in offspring body weight were reported in one avian reproduction study). Therefore, there is no convincing evidence of a potential interaction with the estrogen pathway for glyphosate.

Tier 1 in vitro assays showed no evidence of glyphosate interacting with the androgen pathway via androgen-receptor binding, and glyphosate was negative in an androgen-receptor transactivation assay (Kojima et al., 2004; Kojima, Takeuchi & Nagai, 2010). However, evidence for the aromatase and steroidogenesis assays is conflicting: these were negative for glyphosate alone in the USEPA evaluation and a murine in vitro model (Forgacs et al., 2012), but positive for the coformulants in another laboratory (Benachour et al., 2007; Defarge et al., 2016), with mechanistic underpinning via both the regulatory steroidogenic acute regulatory protein (StAR) and the P450scc cleavage enzyme first shown by Walsh et al. (2000).

The in vivo Tier 1 FSTRA and mammalian assays (i.e. Hershberger) and male pubertal assays were negative in the absence of overt toxicity. The only treatment-related effects observed in the Part 158 mammalian studies in the absence of overt toxicity were decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial separation at 1234 mg/kg bw per day in the post-1998 two-generation reproduction study in rats (the EDSP Tier 2 study). Both effects were observed at a dose that was above the limit dose (1000 mg/kg bw per day) for those studies. No androgen-related effects were seen in the wildlife Part 158 studies (decreases in offspring body weight observed in one avian reproduction study).

For the thyroid pathway, there was no convincing evidence of potential interaction of glyphosate. There were no treatment-related effects on thyroid hormones (thyroxine [T4] and thyroid-stimulating hormone [TSH]), thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity; nor were there any thyroid-related effects observed in the female pubertal assay. In the amphibian metamorphosis assay, there were no developmental effects or alterations in thyroid histopathology. No thyroid-related effects were noted in any of the Part 158 studies.

There is little information about any endocrine-mediated effects of glyphosate, for example, in relation to retinoids, vitamin D receptors, metabolic syndrome, obesogens, glucocorticoids, etc., which is a major data gap. In nonmammalian models, two endocrine-relevant pathways have been reported: retinoic-acid dysfunction was observed in tadpoles exposed to glyphosate formulation, whereas inhibition of cortisol response in fish by selected pesticides was notable in an academic (non-industry funded) report because glyphosate did *not* present a stress response inhibition, unlike most of the other test pesticides (Koakoski et al., 2014). Mechanistic information on the induction of receptors such as aryl hydrocarbon receptor (Takeuchi et al., 2008; Kojima, Takeuchi & Nagai, 2010), peroxisome proliferator-activated receptors (Vainio et al., 1983; Takeuchi et al., 2008; Kojima, Takeuchi & Nagai, 2010) and pregnane X receptor (PXR) (Kojima, Takeuchi & Nagai, 2010) are all negative. While glyphosate was not included in the recent Toxcast screens due to solubility issues, some of the coformulants were, with positive results noted for FD&C Blue No. 1 in some of the endocrine end-points.

Adverse endocrine effects due to glyphosate poisoning in humans have not been reported by poison centres (Bradberry, Proudfoot & Vale, 2004; Kamijo, Takai & Sakamoto,. 2016).

(f) EDSP studies

In vitro assays

Androgen-receptor binding

In an in vitro androgen-receptor competitive binding assay, the binding of a single concentration (1 nmol/L) of [^{3}H]-R1881 (reference androgen) in the presence of increasing concentrations (10^{-10} to 10^{-3} mol/L) of glyphosate (purity 95.93%) was measured. Sprague Dawley rat ventral prostate cytosol was the source of the androgen receptor for the study. Low-salt TEGD buffer (which consists of tris hydrochloride or tris base, ethylenediaminetetraacetic acid, glycerol and dithiothreitol) was used as the vehicle. Altogether three runs were performed, each including dexamethasone as a weak positive control and R1881 as the ligand reference standard.

The saturation binding curves showed a dissociation constant (K_d) for [^{3}H]-R1881 of 0.613 (± 0.041) nmol/L and an estimated maximum amount of binding (B_{max}) of 0.817 (± 0.049) fmol per 100 µg protein for the batch of prostate cytosol used in the study. In the competitive binding runs, the estimated mean log IC_{50} for R1881 (strong positive control) was 9.0 mol/L and for the weak positive control (dexamethasone) was –4.6 mol/L; the mean relative binding affinity for the weak positive control, dexamethasone, was 0.004%. At glyphosate concentrations of 10^{-10} to 10^{-3} mol/L, specific binding of [^{3}H]-R1881 was 92.4–101.3% with the exception of one concentration (10^{-9} mol/L) in run 1, which had an average binding of 66.5%. Review of the data indicated that this value was a result of a single replicate with a specific binding of 7.5%. Excluding this value yielded a mean specific

binding of 96.0%, which concurs with the other runs. Since the specific binding was greater than 75% at all concentrations of glyphosate in all runs, no IC_{50} or relative binding affinity values were estimated. Based on the results from the three runs, glyphosate does not competitively bind to the androgen receptor (Willoughby, 2012a).

Estrogen-receptor binding

In an estrogen-receptor binding assay, the binding of a single concentration of [^{3}H]-17β-estradiol (1 nmol/L) in the presence of increasing concentrations (10^{-10} to 10^{-3} mol/L) of glyphosate (purity 95.93%) was measured. TEGD buffer was used as the solvent vehicle for glyphosate. A total of three runs was performed, each including 19-norethindrone as a weak positive control, octyltriethoxysilane as a negative control and 17β-estradiol as the natural ligand reference chemical.

The K_d for [^{3}H]-17β-estradiol was 0.331 (± 0.061) nmol/L and the estimated B_{max} was 74.55 (± 3.03) fmol per 100 µg protein for the prepared rat uterine cytosol. The K_d for each run was within the expected range of 0.03–1.5 nmol/L. In the competitive binding experiment, the estimated mean log IC_{50} for 17β-estradiol was −9.0 mol/L and for 19-norethindrone was −5.5 mol/L. The mean relative binding affinity was 0.032% for 19-norethindrone, compared to the natural ligand. Glyphosate was tested over a concentration range (10^{-10} to 10^{-3} mol/L) that fully defined the top of the curve. Across all runs, the lowest average per cent radiolabelled estradiol binding in the presence of glyphosate was greater than 81% (i.e. showed less than 25% displacement) at concentrations up to 10^{-3} mol/L. Based on the results from the three runs, glyphosate does not competitively bind to the estrogen receptor (Willoughby, 2012b).

Estrogen receptor transcriptional activation

In an estrogen receptor transcriptional activation (ERTA) assay, hERα-HeLa-9903 cells cultured in vitro were exposed to glyphosate (purity 85.14%) at logarithmically increasing concentrations from 10^{-10} to 10^{-3} mol/L in cell culture media for 24 hours in three independent runs. The experiments were performed using 96-well plates, and each glyphosate concentration was tested in six wells/plate in each run. The solvent vehicle was the culture media for glyphosate and DMSO (0.1%) for the reference chemicals. Cells were exposed to the test agent for 24 (± 2) hours to induce reporter (luciferase) gene products. Luciferase expression in response to activation of the estrogen receptor was measured using a luciferase assay.

Glyphosate was tested up to the limit dose, with no precipitation or cytotoxicity observed at any tested concentration. At concentrations up to 10^{-3} mol/L, the relative transcriptional activation of glyphosate was less than or equal to 2.4%. Glyphosate was only able to reach a maximum of 0.8–2.4% of the positive control, 1 nmol/L 17β-estradiol, when tested up to the highest concentration. Because the RPC_{max} (maximum level of response induced by a test chemical, expressed as a percentage of the response induced by the positive control) was less than the PC_{10} (concentration of a test chemical at which the response is 10% of the response induced by the positive control in both assay runs), glyphosate was considered negative for estrogen receptor transcriptional activation in this test system (Willoughby, 2012c).

Aromatase

Glyphosate (purity 95.93%) was evaluated for its potential to inhibit aromatase activity by incubating with human recombinant aromatase and tritiated androstenedione ([1β-^{3}H(*N*)]-androstene-4-ene-3,17-dione; [^{3}H]ASDN) at log concentrations of 10^{-10} to 10^{-3} mol/L glyphosate. The solvent vehicle was 0.1 mol/L phosphate buffer for glyphosate, ethanol for ASDN and DMSO for 4-hydroxyandrostenedione (4-OH ASDN), with a final assay volume of less than or equal to 1% DMSO. Aromatase activity was determined by measuring the amount of tritiated water produced at

the end of a 15-minute incubation for each concentration of chemical. Tritiated water was quantified using liquid scintillation counting. Each run included a full activity control, a background activity control, a positive control series (10^{-10} to 10^{-5} mol/L) with a known inhibitor (4-OH ASDN) and the test chemical series (10^{-10} to 10^{-3} mol/L) with three repetitions per concentration.

Aromatase activity in the full activity controls was 0.676 (± 0.072) nmol·mg-protein^{-1}·min^{-1}. The response of each full activity control within a run was between 90% and 110% of the average full activity. Activity in the background controls ranged from 0.23% to 0.38% and averaged 0.30% of the full activity control. For the positive control substance (4-OH ASDN), the estimated log IC_{50} averaged −7.29 mol/L and the Hill slope was −0.96. For glyphosate, aromatase activity averaged 0.673 (± 0.066) nmol·mg-protein^{-1}·min^{-1} at the lowest tested concentration of 10^{-10} mol/L and 0.741 (± 0.100) nmol·mg-protein^{-1}·min^{-1} at the highest tested concentration of 10^{-3} mol/L. The average aromatase activity was greater than or equal to 99.67% of the control at all tested glyphosate concentrations for all runs. The results indicate that glyphosate does not inhibit aromatase activity (Wilga, 2012).

Steroidogenesis

The purpose of this study was to validate the use of a standardized steroidogenesis assay as detailed in OECD Guideline for the Testing of Chemicals: Draft Proposal for a New Guideline 4XX – The H295R Steroidogenesis Assay. In this validation study, 28 chemicals were selected as a screen for potential effects of endocrine-disrupting chemicals on the production of testosterone and 17β-estradiol. These chemicals were selected based on their known or suspected endocrine activity, or lack thereof, and included inhibitors and inducers of different potencies as well as positive and negative controls. In this steroidogenesis assay, H295R cells cultured in vitro in 24-well plates were incubated with glyphosate (purity and lot no. not provided) at seven concentrations between 0.0001 and 100 µmol/L for 48 hours in triplicate for three independent experiments. A quality control plate was run concurrently with each independent run of a test chemical plate to demonstrate that the assay responded properly to positive control agents at two concentrations; positive controls included the known inhibitor (prochloraz) and inducer (forskolin) of estradiol and testosterone production. Testosterone and 17β-estradiol levels were measured using radioimmunoassays or enzyme-linked immunosorbent assay (ELISA); responses of the quality control plates measured by these assays were confirmed by liquid chromatography–mass spectrometry. In this validation study, the laboratories demonstrated that glyphosate does not affect testosterone or estradiol levels via this assay (Hecker et al., 2011).

In vivo assay

Hershberger assay

To screen for potential anti-androgenic activity, glyphosate in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six 54- or 55-day old, castrated male Sprague Dawley rats at concentrations of 0 (vehicle), 100, 300 or 1000 mg/kg bw per day with a daily dose of reference androgen testosterone propionate at 0.2 mg/kg bw per day by subcutaneous injection. The anti-androgenic positive control group consisted of six castrated rats exposed to 0.2 mg/kg bw per day testosterone propionate by subcutaneous injection and 3 mg/kg bw per day flutamide via oral gavage. Testosterone propionate alone was used as the anti-androgenic negative control. For both components of the assay, body weights were determined daily. The animals were dosed for 10 consecutive days and terminated approximately 24 hours after the final dose. At necropsy, the five androgen-dependent tissues were collected and weighed.

In the androgen-agonist assay, there were no treatment-related effects on body weights, overall body-weight gains or the weights of accessory sex organs for any glyphosate dose group. Animals in the positive testosterone propionate control group had increased ($P < 0.01$) accessory sex organ weights as follows: 437% in seminal vesicles; 728% in the ventral prostate; 200% in levator

ani-bulbocavernosus; 361% in the Cowper gland; and 45% in the glans penis. The performance criteria indicated that this assay was performing as expected.

In the anti-androgen assay, there were no treatment-related effects on body weights, overall body-weight gains or the weights of accessory sex organs for any glyphosate dose group. Animals dosed with testosterone propionate plus flutamide (positive control) had decreased ($P < 0.01$) accessory sex organ weights as follows: 76% in seminal vesicles; 80% in ventral prostate; 63% in the levator ani-bulbocavernosus; 70% in the Cowper glands; and 29% in glans penis. The performance criteria indicated that this assay was performing as expected.

Statistically significant changes were not seen in two or more of the five androgen sensitive tissue weights. Glyphosate was negative for androgenicity and anti-androgenicity in the Hershberger assay (Stump, 2012a).

Uterotrophic assay

In a uterotrophic assay conducted to screen for potential estrogenic activity, glyphosate (purity 85.14%) in 0.5% methylcellulose (w/v) was administered daily via oral gavage to groups of six ovariectomized female Sprague Dawley rats at dose levels of 0 (vehicle), 100, 300 or 1000 (limit dose) mg/kg bw per day on postnatal days 66/67 to 68/69. The positive control group was treated with a daily dose of 17α-ethynyl estradiol at 3 µg/kg per day by oral gavage. Body weights were determined daily. All the animals were terminated and necropsied approximately 24 hours after the final dose was administered on postnatal day 69/70 to determine wet and blotted uterine weights.

All the animals survived until scheduled termination and no treatment-related clinical findings were observed in glyphosate-dosed animals. Body weights, body-weight gains and uterine weights in the glyphosate groups were comparable to the vehicle control. As expected, absolute wet and blotted uterus weights were increased by 758% and 256%, respectively, in the positive control (17α-ethynyl estradiol) group.

The conclusion reached was that glyphosate was negative in the uterotrophic assay (Stump, 2012b).

Male pubertal assay

In a male pubertal assay, 15 Crl:CD(SD) male rats per dose group were treated daily via oral gavage (5 mL/kg) with glyphosate (purity 95.93%) in 0.5% methylcellulose at 0, 100, 300 or 1000 mg/kg bw per day (limit dose) from postnatal day 23–53. The animals were examined for preputial separation daily beginning on postnatal day 30, and age and weight at day of attainment were recorded. Following termination on postnatal day 53, blood was taken for total thyroxine, testosterone, TSH and clinical chemistry analysis. The hormones were analysed by radioimmunoassay or chemiluminescence.

Treatment-related clinical findings were limited to rales approximately 4 hours post dosing in 9/15 rats at 300 mg/kg bw per day and 14/15 rats at 1000 mg/kg bw per day. This finding persisted in the daily examinations in seven high-dose males throughout the study. On postnatal day 53, final body weights in the 300 and 1000 mg/kg bw per day groups were decreased ($P < 0.05$) by 7–10%. A treatment-related delay in the mean age of attainment of complete preputial separation was noted at 1000 mg/kg bw per day (48.0 days) compared to controls (45.9 days). However, it was determined that this delay at this dose was a result of the treatment-related decrease in body weight, rather than a direct anti-androgenic effect. No treatment-related effects on organ weights were observed at any dose. No treatment-related effects on T4, TSH or testosterone levels were observed at any dose. At 1000 mg/kg bw per day, there was a slight increase in the number of animals with thyroid colloid area grade 4 (five treated vs one control) and grade 5 (one treated vs zero controls). There were no treatment-related effects on follicular cell height at any dose compared to controls; nor were there any treatment-related findings in the testes, epididymides or kidneys.

In conclusion, glyphosate did not affect maturation and did not produce any thyroid toxicity at doses up to 1000 mg/kg bw per day (Stump, 2012c).

Female pubertal assay

In a female pubertal assay, 15 Crl:CD(SD) Sprague Dawley rats/dose group were treated daily via oral gavage with glyphosate (purity 95.93%) in 0.5% methylcellulose at doses of 0, 100, 300 or 1000 mg/kg bw per day (limit dose) from postnatal day 22–42. The animals were examined daily for vaginal opening beginning on postnatal day 22, and age and weight at day of attainment were recorded. Following termination on postnatal day 42, blood was collected for clinical chemistry analyses, including electrochemiluminescent immunoassay (to analyse total thyroxine) and a magnetic [125I]rTSH gamma counter immunoassay (to analyse TSH).

One animal in the control group was terminated in extremis on postnatal day 27 due to impairment of the right forelimb (due to possible mechanical injury). There were no treatment-related differences in age of attainment of vaginal opening, body weights at vaginal opening, final body weights or body-weight gains in the treated groups relative to controls. One control female and one at 300 mg/kg bw per day failed to attain vaginal opening. There were no statistically significant differences in mean age at first vaginal estrus, mean cycle length or per cent cycling. The cycle status at necropsy was similar across all groups. Serum total thyroxine and TSH concentrations were not affected by treatment, and no adverse treatment-related effects on any clinical chemistry parameter were observed at any dose. There were no treatment-related microscopic findings in the thyroid, ovaries, uterus or kidneys at any dose.

In conclusion, glyphosate did delay the maturation and no treatment-related effects were seen in thyroid toxicity (Stump, 2012d).

Additional literature reports

The published literature was reviewed and is included with the EDSP data, in the summary Table 47.

Estrogen pathway

With in vitro studies of estrogen receptor activation, Thongprakaisang et al. (2013) reported estrogen receptor agonism by glyphosate at concentrations from 10^{-12} to 10^{-6} mol/L in estrogen-dependent human breast cancer cells, but did not test the estrogen receptor α antagonism as recommended by the test developers (Evans, Gray & Wilson, 2012). In contrast, other studies reported negative results in reporter gene–transfected Chinese hamster ovary (CHO) cells (Kojima et al., 2004; Kojima, Takeuchi & Nagai, 2010) or that glyphosate formulations reduced the transcription of estrogen receptor α and estrogen receptor β in HepG2 cells transiently transfected with the reporter gene ERE, but the glyphosate parent did not (Gasnier et al., 2009).

In an in vivo rainbow trout VTG assay, glyphosate did not increase plasma VTG in juvenile rainbow trout, and plasma VTG levels in glyphosate plus surfactant–treated trout were only marginally greater than the controls, with no trend and no significance (Xie et al., 2005).

In conclusion, there is no convincing evidence of a potential interaction with the estrogen pathway for glyphosate. The one positive in vitro study has not been reproduced by another laboratory.

461

Androgen pathway

The Séralini laboratory conducted several androgen pathway–related assays in equine testes utilizing principally non-validated in vitro assays as well as ex vivo assays. These suggested effects of glyphosate for anti-androgenicity and inhibition of aromatase activity (Richard et al., 2005; Benachour et al., 2007; Gasnier et al., 2009; Defarge et al., 2016). Studies in other laboratories did not report this (Kojima et al., 2004; Kojima, Takeuchi & Nagai, 2010) and particularly those that used the EDSP battery of validated tests for the androgen receptor–mediated and steroidogenesis.

The differences observed in the in vitro studies with positive results and those with negative results may be due to confounding by the glucocorticoid receptor interference in the cell line used in the non-validated assays; the MDA-MB453-kb2 cell line has a high glucocorticoid-receptor content in addition to androgen-receptor content.

Additional steroidogenic mechanisms of interest include a noted effect upon the post transcriptional expression of the StAR in mouse testicular Leydig cells (Walsh et al., 2000) This was also reported for P450scc, the enzyme responsible for the conversion of cholesterol to pregnenolone and for initiating the synthesis of all steroid hormones cells (Walsh et al., 2000). However, another in vitro Leydig cell model reported no effect of glyphosate on basal or recombinant human chorionic gonadotrophin (rhCG) (Forgacs et al., 2012).

In conclusion, there is no convincing evidence of a potential interaction between glyphosate and the androgen receptor pathway. Decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial separation (at 1234 mg/kg bw per day in the two-generation reproduction study in rats) were observed at a dose that was above the limit dose (1000 mg/kg bw per day), and therefore of low physiological relevance.

However there is plausible but equivocal evidence that glyphosate and glyphosate coformulants affect the steroidogenesis pathway, via P450scc and StAR. This requires further investigation.

Thyroid pathway

No relevant in vitro or mammalian in vivo reports on the effect of glyphosate on the thyroid pathway were identified in the literature, and the EDSP data had no evidence.

A handful of reports describe the effect of glyphosate on the negative metamorphosis of frog and tadpole species, including a 2014 report that identified alterations in genes encoding thyroid hormone receptor beta in brain, glucocorticoid receptor in tail and deiodinase enzyme in brain and tail (Lanctot et al., 2014), suggesting that glyphosate formulations have the potential to alter mRNA profiles during metamorphosis.

Other endocrine-related pathways

Following studies conducted in *Xenopus laevis* and chicken embryos, the retinoic acid–signalling pathway has been proposed as a mechanistic pathway that is adversely affected by glyphosate (Paganelli et al., 2010). In this study, a 1/5000 dilution of glyphosate induced reproducible skeletal and craniofacial malformations. Developmental toxicity studies in the rabbit (Section 2.5b, Rabbits) identified nonsignificant skeletal malformations, with the lowest NOAEL for developmental toxicity 250 mg/kg bw per day (Bhide & Patil, 1989). The NOAEL and LOAEL for this study are based on the available data (Bhide & Patil, 1989) as individual data were not provided. A subsequent study NOAEL of 300 mg/kg bw per day was based on delayed ossification and an increased incidence of fetuses with skeletal anomalies at 1000 mg/kg bw per day (Brooker et al., 1991a). However, these effects were secondary to the observed severe maternal toxicity. Nevertheless, the retinoic-acid pathway constitutes a data gap that requires further research.

Other receptor-mediated pathways reported in the literature, including aryl hydrocarbon receptors and peroxisome proliferator-activated receptors, were negative.

462

Cortisol stress pathways

A study of the stress response of *Rhamdia que/en* fingerlings with acute exposure to a glyphosate formulation (360 giL) at 45, 90, 135 and 180 days did not demonstrate impairment of cortisol release but did exert negative effects on growth and survival parameters (Koakoski et a!., 2014).

Table 47. Summary of information supporting EDSP data in relation to glyphosate and endocrine end-points

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Estrogen pathway						
EDSP Tier 1 data 2014/2015: In vitro: ER binding; TG 455 ER STTA and Hela Assay In vivo: mammalian assays, i.e. uterotrophic and female pubertal assays and mammalian toxicity studies	Glyphosate Purity: 85.1–95.93% Concentration range: 10^{-10} to 10^{-3} mol/L	USEPA validated assays In vitro assays are well-characterized and OECD TGs ER STTA: uses HeLa cell line which has ER α not ER β. ER α perturbation is more strongly associated with adverse outcomes	There were no treatment-related effects on female reproductive parameters in the existing glyphosate Part 158 mammalian or wildlife studies, however decreases in offspring body weight were observed in one avian reproduction study	High	Negative	USEPA (2015)
In vitro: ER agonism in estrogen-dependent T47D human breast cancer cells	Glyphosate Purity > 98%) Accustandard Concentration range: 10^{-12} to 10^{-6} mol/L	Validated assay	Glyphosate exerted proliferative effects only in human hormone-dependent breast cancer, T47D cells, and not in hormone-independent breast cancer, MDA-MB231 cells, at 10^{-12} to 10^{-6} mol/L in estrogen withdrawal condition, which was reported to be confirmed by the inhibitory effect of the ER antagonist ICI 182780. The T47D cell line contains both ERα and ERβ. While the use of ICI 182780 can exclude the possibility of dioxin-like interference of coformulant contaminant 1,4-dioxane with AhR interactions affecting the ER, this study is confounded because it was not tested with an ERα-specific antagonist, such as methylpiperidino pyrazole (CAS No. 289726-02-9). This would determine the relative activities of each ER (Evans, Gray & Wilson, 2012) The luciferase reporter system was then also used with combinations of genistein, an isoflavone in soy. Phytoestrogens such as genistein are known to overstimulate luciferase, and also are stronger ligands for ERβ. Non-receptor–mediated luminescence signals have	Low	Positive	Thongprakaisang et al. (2013)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			been reported at phytoestrogen concentrations higher than 1 µmol/L due to the over-activation of the luciferase reporter gene (Kuiper et al., 1998; Escande et al., 2006). While the dose–response curve indicates that true activation of the ER system occurs at lower concentrations, luciferase expression obtained at high concentrations of phytoestrogens or similar compounds suspected of producing phytoestrogen-like over-activation of the luciferase reporter gene needs to be examined carefully in stably transfected ERTA assay systems. (See Annex 2 of OECD TG 455)			
In vitro: hERα and hERβ (ant)agonism in reporter gene–transfected CHO cells	Glyphosate (> 95–100%); whether this is a formulation is not specified in the paper. Concentrations for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.		Concentrations of pesticides that tested negative, which included glyphosate, are not reported; only the results of those that tested positive are provided. Concentration of positively testing chemicals ranged from 10^{-6} to 10^{-12} mol/L Concentration of the test chemicals showing 20% of the agonistic activity of 10^{-10} mol/L 17β-estradiol, and is given as REC 20(mol/L)	Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hERα and hERβ transient transfection into human hepatocarcinoma HepG2 cells	Glyphosate formulations and glyphosate parent chemical. Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line	Formulations reduced transcription of *ERα* and *ERβ* in HepG2 cells transiently transfected with ERE, but glyphosate parent did not	Med	Parent-negative Formulations-positive	Gasnier et al. (2009)
In vivo: FSTRA		In this validated assay, the non-treatment-responsive decrease (only significant at mid-treatment) in VTG was seen in isolation in the absence of any treatment-related effects in the other estrogen-related end-points such as gonado-somatic		Med	Negative	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
		index, gonadal staging, fecundity and fertilization. In addition, there were no notable gonadal histopathology				
In vivo: Rainbow trout VTG assay	Glyphosate and glyphosate plus surfactants; measured concentration of glyphosate 0.11 mg/L for 7 days	VTG induction in fish is a standard measure for estrogenicity in environmental regulatory toxicology that also considers the relevance to humans (e.g. USEPA FIFRA SAP 2009a,b, 2012). Glyphosate did not increase plasma VTG in levels in juvenile rainbow trout, glyphosates plus surfactants were only marginally greater than the controls, no trend, no significance		Med	Negative	Xie et al. (2005)
Overall conclusion: No convincing evidence of a potential interaction with the estrogen pathway. The one in vitro study that is positive has not been reproduced by another laboratory.						
Androgen pathway						
EDSP Tier 1 data 2014/2015: In vitro: negative; both for androgen-receptor binding assay and the aromatase assay In vivo mammalian assays: Hershberger and male pubertal assays	Glyphosate	Standardized and validated assays	Androgen-receptor binding assay is not a validated OECD TG but other validated androgen receptor assays not available in 2014/2015 Aromatase assay: highest soluble test concentration of glyphosate was 10^{-3} mol/L The in vivo Tier 1 FSTRA and mammalian assays (i.e. Hershberger and male pubertal assays) were negative in the absence of overt toxicity. The only treatment-related effects observed in the Part 158 mammalian studies in the absence of overt toxicity were decreases in sperm count in the subchronic rat study (1678 mg/kg bw per day) and a delay in preputial	High	Negative, but sperm count and delay in preputial separation effects seen at very high doses, > 1 000 mg/kg bw per day	USEPA (2015)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
			separation at 1 234 mg/kg bw per day in the post-1998 two-generation reproduction study in rats (the EDSP Tier 2 study). Both effects were observed at a dose that was above the limit dose (1 000 mg/kg bw per day) for those studies. No androgen-related effects were seen in the wildlife Part 158 studies (decreases in offspring body weight observed in one avian reproduction study)			
In vitro: hAR transactivation assay in CHO cells	Glyphosate (> 95–100%) formulation not specified in the paper Concentrations given for glyphosate are not clearly specified, but can be assumed to be the same as those for the positive chemicals.			Med	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
In vitro: hAR transient transfection into human HepG2 cells, aromatase evaluation within the HepG2 cells, and MDA-MB453-kb2 cells	Glyphosate and formulations Dilutions up to 10^{-7}	Non-validated assays, but well-recognized and reliable hepatic cell line. Method for aromatase activity evaluation is also part of OECD TG 456 for steroidogenesis.	MDA-MB453-kb2 cell line has a high content of glucocorticoid receptors in addition to androgen receptors The characterization of the cell line and discussion of such confounding factors is not considered in the paper. While glyphosate and formulations reduced AR transcription in this cell line, there appears to have been no control with androgen-specific responses to exclude glucocorticoid-specific responses	Low	Positive	Gasnier et al. (2009)
Steroidogenesis In vitro: Transformed and human aromatase–transfected cDNA in human embryonic kidney 293 cells and placental-	Glyphosate and formulations 0.01% (with 210 µmol/L glyphosate) to 2% glyphosate/glyphosate formulation	Relevant cell models, but limited characterization provided in the paper	Inhibition of aromatase noted in two different species by both parent compound and formulations The aromatase assay may be subject to variability, e.g. due to degradation of the enzyme, and therefore performance criteria are specified in guideline OPPTS 890.1200 to	Low–Med	Positive	Benachour et al. (2007)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
derived JEG3 cells Ex vivo: normal human placenta and equine testis			demonstrate that the assay is functioning correctly. This is addressed in the EDSP data, but is not evident in the Séralini lab. papers (Benachour et al., 2007; Gasnier et al., 2009), although OECD GD 150 is cited. An adequate response with the proficiency chemicals econazole, fenarimol, nitrofen (inhibitors) and atrazine (non-inhibitor) should be demonstrated and the inhibitor 4-hydroxyandrostenedione (formestane) used as a positive control chemical in each experiment. While the correct positive control was used, proficiency testing is not reported Compliance with the performance criteria should be checked before evaluating results from this assay. A positive result in GD OPPTS 890.1200 requires demonstration of inhibition of aromatase activity that fits a 4-parameter nonlinear regression model such that the concentration response curve crosses 50% inhibition. The concentration response curve allows the determination of potency, i.e. IC_{50}. In some cases, variability may be due to limited solubility of a chemical			
Steroidogenesis In vitro: Placenta-derived JEG3 cells	Glyphosate and formulation ingredients Top dose: 100 ppm		The coformulants were each tested independently and were reported to inhibit aromatase activity at concentrations 20–67% below the no-observed-effect concentration, at which levels glyphosate alone did not significantly inhibit aromatase. (See also comment above regarding proficiency testing of the assay)	Low–Med	Positive	Defarge et al. (2016)
In vitro: BLTK1 murine Leydig cells	300 µmol/L	characterized Leydig cell model				(2012)

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Steroidogenesis In vitro: StAR in a mouse MA-10 Leydig tumour cell	Glyphosate formulation (containing 180 g/L glyphosate)	Relevant and well-characterized cell model	Statistically significant reduction ($P < 0.01$) of $(Bu)_2$cAMP with the glyphosate formulation was observed after 2 hours of treatment. Statistical significance ($P < 0.01$) was also [illegible] for the conversion of cholesterol to pregnenolone and for initiating the synthesis of all steroid hormones	Med–High	Positive	Walsh et al. (2000)
Overall conclusion: There is no convincing evidence of a potential interaction between glyphosate and the androgen-receptor pathway. Decreases in sperm count in the subchronic rat study (1 678 mg/kg bw per day; USEPA 2015) and a delay in preputial separation at 1234 mg/kg bw per day in the two-generation reproduction study in rats (the EDSP Tier 2 study) were observed at a dose that was above the limit dose (1000 mg/kg bw per day) and therefore of low physiological relevance. However, there is plausible evidence that glyphosate and glyphosate coformulants affect the steroidogenesis pathway, via P450scc and StAR. Further investigation is needed.						
Thyroid						
EDSP Tier 1 data 2014/2015: In vitro: No assays conducted. In vivo test battery: There were no treatment-related effects on T4 and TSH, thyroid weights or thyroid histopathology in the male pubertal assay in the absence of overt toxicity. No thyroid-related effects were observed in the female pubertal assay. There were no developmental effects or alterations in thyroid histopathology in the amphibian metamorphosis assay. No thyroid-related effects were noted in any of the Part 158 studies.	Glyphosate	Relevant and validated test methods	No convincing evidence of potential interaction of glyphosate	High	Negative	USEPA 2015

End-point pathway	Glyphosate formulation	Strengths	Uncertainties/considerations	Influence on conclusion[a]	Reference conclusion	Reference
Overall conclusion: There is no convincing evidence of a potential interaction with the thyroid pathway for glyphosate						
Other endocrine mechanisms						
Retinoid system In vivo *Xenopus laevis* embryo model and chicken embryos	360 pg and 5 000 pg of glyphosate (Sigma)	Whole vertebrate models, two species	Experimental design and hypothesis based on medical observations of craniofacial defects with malformations observed in humans residing in areas chronically exposed to glyphosate formulations. Suspected to be resulting from a dysfunctional retinoic-acid or Sonic hedgehog pathway. Further investigation is needed	Med–High	Positive: increase in endogenous retinoic-acid activity	Paganelli et al. (2010)
Cortisol In vivo fish study *Rhamdia quelen* fingerlings	Glyphosate formulation 360 g/L	Stress response of *Rhamdia quelen* fingerlings acute exposure at 45, 90, 135 and 180 days	Stress responses important but difficult variable to control for, as stress is induced from handling, etc. This study included appropriate controls for stress confounders	Med	Negative for impaired cortisol release, but impaired growth and survival	Koakoski et al. (2014)
Hypolipidaemia and peroxisome proliferation In vivo rat	Glyphosate formulation 300 mg/kg single daily dose for 2 weeks, 5 animals/dose per group		No increase in number or size or peroxisomes	Med	Negative	Vainio et al. (1983)
AhR induction In vitro: Mouse hepatoma Hepa1c1c7 cells AhR Luciferase reporter gene transcriptional assay	Glyphosate (95–100% purity) Assay performed at concentrations of $\leq 10^{-5}$ mol/L	Relevant and recognized assay		Med	Negative	Takeuchi et al. (2008)
In vitro mPPARα, mAhR, hPXR	Glyphosate		Review, insufficient detail given. Concentration tested not given for negative test chemicals	Low	Negative	Kojima et al. (2004); Kojima, Takeuchi & Nagai (2010)
Overall conclusion: Suggestion of adverse effect upon retinoic-acid pathways. Further investigation required.						

AhR: aryl hydrocarbon receptor; AR: androgen receptor; CAS: Chemical Abstracts Service; CHO: Chinese hamster ovary; EDSP: Endocrine Disruptor Screening Program; ER: estrogen receptor; ERTA: estrogen receptor transcriptional activation; FSTRA: fish short-term reproduction assay; GD: guideline; hAR: human androgen receptor; HepG2: hepatocellular carcinoma; IC_{50}: median inhibitory concentration; no.: number; OECD: Organisation for Economic Co-operation and Development; PPAR: peroxisome proliferator-activated receptor; PXR: pregnane X receptor; rhCG: recombinant human chorionic gonadotrophin; StAR: steroidogenic acute regulatory protein; T4: thyroxine; TG: test guideline; TSH: thyroid-stimulating hormone; VTG: vitellogenin

[a] High: line of evidence could be sufficient on its own to be almost sure of entry (approaching 100% likelihood); Med: contributes importantly towards increasing likelihood; Low: minor contribution towards increasing likelihood.

(g) Microbiological effects

Bacteria

The herbicidal action of glyphosate is generated by chelating manganese required in the reduction of the flavin mononucleotide cofactor 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) (Cerdeira & Duke, 2006). Since bacteria have EPSPS and produce amino acids via the shikimate pathway, there is potential for glyphosate residues to disrupt microbes in the human gastrointestinal tract. However, no studies have specifically addressed whether glyphosate affects the microbiota in the human gastrointestinal tract or in mouse and rat animal models. What is known is that selected bacterial pathogens and probiotic bacteria from dairy cows and poultry can be affected differently by residual levels of glyphosate.

The minimum inhibitory concentration (MIC) of glyphosate on the growth and viability of poultry microbiota and pathogens was determined in triplicate in 24-well microtitre plates. Just 100 µL of the tested bacteria (105 colony-forming units [cfu] per mL) was added to 900 µL broth media containing different concentrations of glyphosate (0.075, 0.15, 0.30, 0.60, 1.20, 2.40 or 5.0 mg/mL). Plates containing glyphosate and bacteria were incubated at 37 °C. MIC values were determined by quantitative analysis of bacteria on agar plates.

Clostridium perfringens, Salmonella gallinarum, S. typhimurium and S. enteritidis were highly resistant to glyphosate (MIC of 5 mg/mL). *Lactobacillus casei, L. buchneri, L. harbinensis, Staphylococcus aureus, S. lentus* and *S. haemolyticus* were moderately resistant to glyphosate (MIC 0.60–0.30 mg/mL). All other tested bacteria including *Enterococcus faecalis, E. faecium, Bacillus badius, B. cereus* and *Bifidobacterium adolescentis* were highly sensitive to glyphosate, with MIC values ranging from 0.15 to 0.075 mg/mL (Table 48). Pathogenic *E. coli* and *E. coli* 1917 strain Nissle were also found to be resistant to glyphosate (MIC of 1.2 mg/mL).

In summary, most of the tested pathogenic bacteria were highly resistant to glyphosate; however, most other tested bacteria were moderate to highly susceptible (Shehata et al., 2013b).

Table 48. Inhibitory effects of glyphosate on different bacteria

		Bacterial count [a]	
Genus/species	**MIC (mg/mL)**	**Treated with glyphosate at MIC**	**Not treated with glyphosate**
Bacillus badius	0.15	2.24 ± 0.49	8.90 ± 0.44
B. cereus	0.3	2.75 ± 0.68	8.08 ± 0.12
Bacteriodes vulgatus	0.6	3.54 ± 0.31	7.37 ± 0.10
Bifidobacterium adolescentis	0.075	3.87 ± 0.50	8.67 ± 0.48
Campylobacter coli	0.15	3.07 ± 0.50	9.00 ± 0.70
C. jejuni	0.15	3.90 ± 0.50	9.54 ± 0.97
Clostridium perfringens	5.0	3.37 ± 0.89	8.30 ± 0.28
C. botulinum type A	1.2	4.00 ± 0.50	8.16 ± 0.32
C. botulinum type B	1.2	3.56 ± 0.45	7.60 ± 057
E. coli	1.2	3.15 ± 0.24	8.00 ± 0.34
E. coli 1917 strain Nissle	1.2	2.35 ± 0.24	7.26 ± 0.21
Enterococcus faecalis	0.15	2.00 ± 0.45	8.49 ± 0.58
E. faecium	0.15	2.01± 0.34	7.06 ± 0.95
Lactobacillus buchneri	0.6	4.00 ± 0.88	8.00 ± 0.34
L. casei	0.6	4.74 ± 0.56	8.28 ± 0.35
L. harbinensis	0.6	5.30 ± 0.44	8.40 ± 0.32

Genus/species	MIC (mg/mL)	Bacterial count [a]	
		Treated with glyphosate at MIC	Not treated with glyphosate
Riemerella anatipestifer	0.15	4.00 ± 0.50	7.88 ± 0.50
Salmonella enteritidis	5.0	2.35 ± 0.26	8.28 ± 0.16
S. gallinarum	5.0	2.15 ± 0.33	8.68 ± 0.20
S. typhimurium	5.0	2.75 ± 0.68	8.03 ± 0.16
Staphylococcus aureus	0.3	5.74 ± 0.58	9.00 ± 0.10
S. haemolyticus	0.3	5.74 ± 0.32	8.08 ± 0.16
S. lentus	0.3	3.90 ± 0.44	8.08 ± 0.14

MIC: minimum inhibitory concentration; SD: standard deviation

[a] Mean of $n = 3$ quantitative bacterial counts expressed as reciprocal $\log_{10}$ ± SD.

Source: Shehata et al. (2013b)

An evaluation of the effects of Roundup and its glyphosate ingredients on the growth and viability of three food-associated microorganisms widely used as starters in traditional and industrial dairy technologies found that glyphosate inhibited the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* at a concentration of 1 mg/mL and *Lactococcus lactis* subsp. *cremoris*, which was more sensitive to glyphosate, with an MIC of 0.312 mg/mL (Table 49). The fungus *Geotrichum candidum* was more sensitive, with an MIC of 0.100 mg/mL (Clair et al., 2012).

Table 49. Effect of Roundup on three food-associated microorganisms

Microorganism strain	Concentration of glyphosate in Roundup (g/L)	MIC (ppm)	MMC (ppm)
G. candidum ATCC 204307	400	100	1000
	450	625	1000
L. lactis subsp. *cremoris* ATCC 19257	450	312	625
L. delbrueckii subsp. *bulgaricus* CFL1	450	1000	1250

MIC: minimum inhibitory concentration; MMC: minimum microbicidal concentration; ppm: parts per million

MIC and MMC measured after 24-hour incubation in growth media supplemented with Roundup or equivalent amount of glyphosate.

Source: Clair et al. (2012)

The minimal agricultural use of the herbicide is 10 000 ppm.

In a study of the impact of glyphosate on poultry microbiota and the production of botulinum neurotoxin during ruminal fermentation, ruminal microbiota were characterized by fluorescence in situ hybridization technique using 16S rRNA/23S rRNA-targeted oligonucleotide probes. After incubation with 0, 1, 10 or 100 µg/mL glyphosate in rumen fluids from donor cows, the cell counts of *Ruminococcus albus* and *R. flavefaciens* were significantly lower in the presence of 1 µg/mL glyphosate; *Streptococcus* spp. cell counts were significantly lower with 100 µg/mL glyphosate, and cell counts of the phylum Euryarchaeota were significantly lower on exposure to 10 and 100 µg/mL. In contrast, cell counts of *Clostridium histolyticum* and *Lactobacilli* and *Enterococci* were significantly higher with 100 µg/mL glyphosate. The study authors noted that more bacterial species were inhibited when cows were fed a crude fibre-rich diet than a lower-fibre diet, indicating a possible inhibitory effect on the microbiota responsible for fibre degradation (Ackermann et al., 2015).

In a study of the toxicity of glyphosate to the most prevalent *Enterococcus* spp. in the gastrointestinal tract, the lowest concentration of glyphosate and Roundup to show bactericidal or bacteriostatic effects was determined in 96-well microtitre plates. Serial dilutions of glyphosate from
10–0.001 mg/mL were made in nutrient broth. *Enterococcus* isolates were added at a final concentration of 10^4 cfu/mL, and the test plates with diluted glyphosate and *Enterococcus* incubated overnight at 37 °C before plating aliquots on citrate azide tween carbonate agar. Bacterial growth on each agar plate was evaluated.

Glyphosate and Roundup at 0.1–10 mg/mL inhibited the growth of *E. faecalis* but not of *C. botulinum* or the production of botulinum neurotoxin (Table 50). The study authors proposed that glyphosate may be a significant factor in the observed increased risk of *C. botulinum* infection in cattle in Germany over the past 10 to 15 years (Krüger et al., 2013). Glyphosate toxicity to *Enterococcus* spp. leads to an imbalance in the gut favouring overgrowth of *Clostridium* spp. because the common, beneficial bacteria, *Enterococcus* spp., suppress *Clostridium* growth in the gastrointestinal tract (Krüger et al., 2013; Shehata et al., 2013a,b).

***Table 50. Effect of glyphosate and Roundup on the growth of* C. botulinum *type B and* E. faecalis**

Herbicide concentration (mg/mL)	Glyphosate			Roundup formulation		
	C. botulinum type B (cfu/mL) [a]	BoNT (ng/mL) [b]	*E. faecalis* (cfu/mL) [c]	*C. botulinum* type B (cfu/mL) [a]	BoNT (ng/mL)	*E. faecalis* (cfu/mL)
0	6.9 ± 0.34	300 ± 47	8.2 ± 0.87	6.9 ± 0.34	270 ± 120	8.2 ± 0.87
0.1	5.3 ± 0.78	312 ± 20	0	5.1 ± 0.78	337 ± 50	0
1	5.4 ± 0.45	319 ± 60	0	3.3 ± 0.80	0	0
10	3.2 ± 0.43	0	0	3.0 ± 0.65	0	0

BoNT: botulinum neurotoxin; cfu: colony-forming unit; ELISA: enzyme-linked immunosorbent assay; SD: standard deviation

[a] *C. botulinum* type B (10^4/mL) cultured anaerobically in reinforced clostridial medium containing different concentrations of glyphosate or herbicide formulation for 5 days. *C. botulinum* quantified using the most probable number estimation method. Data express as reciprocal $\log_{10}$

[b] *C. botulinum* type B quantified by ELISA.

[c] *E. faecalis* cultured aerobically in reinforced clostridial medium containing different concentrations of glyphosate or herbicide formulation for 8 hours and quantified on citrate-acid-tween-carbonate agar. Data expressed as reciprocal $\log_{10}$ ± SD.

Source: Krüger et al. (2013)

The neutralization ability of the antimicrobial effect of glyphosate by different humic acids was investigated by determining the MIC of glyphosate for different bacteria in different concentrations (0.25, 0.5 and 1.0 mg/mL) of humic acid. The MIC values of glyphosate for *E. faecalis, B. badius and B. adolescentis* were 0.3, 0.3 and 0.15 mg/mL, respectively. Humic acids neutralized the antimicrobial effect of glyphosate in different patterns. The WH67/2, WH67/4/3 and WH67/4 humic acids at 1 mg/mL showed the highest degree of neutralization of the antimicrobial effect of glyphosate. The MIC values of glyphosate for *E. faecalis, B. badius* and *B. adolescentis* in the presence of 1 mg/mL WH67/2, WH67/3, and WH67/4 humic acids were more than 2.4 mg/mL, while the MIC values in the presence of other humic acids ranged from 0.3 to 0.6 mg/mL (Shehata et al., 2014). Sorption of the glyphosate to humic acids varied, depending upon their macromolecular structure, but overall, these compounds neutralized the antimicrobial effect of glyphosate (Piccolo et al., 1995, 1996).

Rats

Toxicokinetics of glyphosate after single 100 mg/kg intravenous and 400 mg/kg oral doses were studied in rats. The oral bioavailability of glyphosate was 23.21% (Anadón et al., 2009). This was lower than the oral bioavailability in studies in which [^{14}C]glyphosate was administered orally at 10 mg/kg, when approximately 30–36% of the dose was absorbed (Howe, Chott & McClanahan, 1988; Ridley & Mirley, 1988; Brewster, Warren & Hopkins, 1991). A National Toxicology Program study showed that approximately 19–23% of the 1000 mg/kg dose was absorbed, as determined from urinary excretion data (Chan & Mahler, 1992). Conversely, when a single oral dose of glyphosate (6–9 mg/kg) was administered to New Zealand White rabbits, 80% of the test material appeared in the faeces (Colvin & Miller, 1973c). Glyphosate is poorly metabolized in rats, and the metabolite AMPA represented 6.49% of the parent drug plasma concentration. A similar metabolic characterization was indicated by Brewster et al. (1991). The production of this metabolite could have been the result of intestinal microbial action (Rueppel et al., 1977; Mueller et al., 1981). Taken together, the fraction of the oral dose of glyphosate bioavailable to intestinal microorganisms could range from 70–80% and be microbiologically active. The microbiological activity of the minor metabolite AMPA has not been determined.

Humans

A review of the published scientific literature found no specific information on whether glyphosate bioaccumulates or affects the microbiota in the human gastrointestinal tract. There are no data that show measurements of the amount of glyphosate residues in human gastrointestinal tract. However, several pharmacokinetic, toxicokinetic and bioavailability studies indicate that glyphosate is poorly absorbed after oral administration.

A review of the literature does not indicate that intestinal bacteria generally found in the human gastrointestinal tract have been tested for the ability to degrade glyphosate. However, the microbial capacity for glyphosate degradation has been shown in terrestrial and aquatic environments (Balthazor & Hallas, 1986; Rueppel et al., 1977; Sprankle, Meggitt & Penner, 1975; Mueller et al., 1981; Franz et al., 1997; Zaranyika & Nyandoro, 1993; Kryuchkova et al., 2014). Glyphosate is metabolized by several bacteria in soil to give sarcosine, which is then converted to glycine and ammonia by sarcosine oxidase. An alternative metabolic pathway involves the formation of AMPA by glyphosate oxidoreductase, which is found in colon tissue in rats (Brewster et al., 1991). Therefore, based on the enzymatic repertoire of the intestinal microbiota, there is potential for these microorganisms to metabolize glyphosate.

There are no specific studies on the effects of glyphosate on the mammalian gut microbiota in mouse, rat, rabbit or humans, that is, there is a lack of in vivo studies: all reports are on in vitro tests. In addition, there are no data on the microbiological activity of the glyphosate metabolites, for example, AMPA.

Many of the chronic and long-term in vivo studies reviewed in this monograph reported that high doses of glyphosate have an impact upon the gastrointestinal tract. While not uncommon with administration of high-dose chemical substances, this merits further investigation as glyphosate is known to be poorly absorbed in mammalian models and alterations in gut microbiota profiles, specifically reductions in the beneficial microbiota and increases in pathogenic bacteria, are known to affect the early initiation and progression of the multistep processes in carcinogenesis (Viljoen et al., 2015).

Evidence from livestock species indicates that pathogenic bacteria are more resistant to glyphosate, while beneficial microbiota are more sensitive, and thus more vulnerable (Shehata et al., 2013b). There is also evidence of intestinal metabolism of glyphosate to AMPA in the colon tissue of rats (Brewster, Warren & Hopkins, 1991).

While plausible mechanistic links could be postulated between chromosome breakage, Bcl-2 and p53, adverse gut microbiome profiles in relation to glyphosate formulations (including the

solvent/contaminant 1,4-dioxane), the (nonsignificant) association seen between glyphosate exposure and non-Hodgkin lymphoma (McDuffie et al., 2001) and mechanisms of action of several proteins closely associated with non-Hodgkin lymphoma (NHL) pathogenesis (Song et al., 2016), there are major knowledge gaps in addressing this question. This is because the available information does not specifically address measurement of glyphosate residues in the (gastro)intestinal tract or whether glyphosate adversely affects the normal functioning of the microbiota in the human gastrointestinal tract or the gastrointestinal tract of experimental mammalian models.

2.7 Studies on metabolites: AMPA

AMPA is the only identified metabolite found in the urine and faeces of orally treated rats. It was reviewed by the JMPR in 1997. The Meeting established an acceptable daily intake (ADI) of 0–0.3mg/kg bw (sum of glyphosate and AMPA) based on a NOAEL of 31 mg/kg bw per day, the highest dose tested in a 26-month study of toxicity in rats with glyphosate.

(a) Acute toxicity of AMPA

Mice

In an acute oral toxicity study, five male and five female ICR(Crj:CD-1) mice were orally dosed with AMPA (purity 99.33%) at 5000 mg/kg bw. The test material was administered as a 25% suspension in 1% CMC sodium solution at 20 mL/kg bw. There were no deaths and no signs of toxicity. All mice gained weight on days 0–7; one male and two females had slight weight losses on days 7–14. There were no observed abnormalities at necropsy.

The oral LD_{50} of AMPA in male and female mice was greater than 5000 mg/kg bw (Komura, 1996).

Rats

In an acute oral toxicity study, five male and five female Wistar-derived Alpk:AP$_f$SD(SPF) albino rats were orally dosed with 5000 mg/kg bw AMPA (assumed purity 100%). The test material was administered as a 50% suspension in 0.5% aqueous polysorbate 80 at a constant dose volume of 10 mL/kg bw.

There were no deaths. Signs of toxicity included diarrhoea, chromodacryorrhea, piloerection, stains around the nose and ungroomed appearance, with recovery by day 5. All the rats but one male gained weight on days 1–8; two males and three females had weight losses on days 8–15. No abnormalities were observed at necropsy.

The oral LD_{50} of AMPA in male and female rats was greater than 5000 mg/kg bw (Leah, 1988).

In a study of acute oral toxicity, five male and five female Sprague Dawley rats were administered AMPA (purity 99.2%) in 0.5% CMC as a single dose at 5000 mg/kg bw by gavage.

Clinical signs, observed 4 hours after dosing, included piloerection, diarrhoea, subdued behaviour, hunched appearance and soiled anal and perigenital areas. All the animals had normal body-weight gain throughout the experiment. No abnormalities were detected at necropsy after 14 days of observation.

The acute oral LD_{50} of AMPA in rats was greater than 5000 mg/kg bw (Cuthbert & Jackson, 1993a).

476

In a study of acute dermal toxicity, five male and five female Sprague Dawley rats were treated with a single 2000 mg/kg bw dose of AMPA (purity 99.2%). The test material was evenly spread on a 5 × 5 cm dressing moistened with distilled water that was then placed on the shaved back of each rat. The patch was covered with an occlusive dressing and kept in contact with the skin for 24 hours. At the end of the exposure period the patch was removed and the exposed skin wiped with distilled water to remove any excess test material.

There were no mortalities after a single dermal application of AMPA at 2000 mg/kg bw and no clinical signs or abnormalities were noted at necropsy. Thus, the acute dermal LD_{50} of AMPA to rats must be greater than 2000 mg/kg bw (Cuthbert & Jackson, 1993b).

In an acute dermal toxicity study, 2000 mg AMPA (purity 98.0%) suspended in 0.5% aqueous hydroxypropylmethylcellulose gel was applied at a volume of 10 mL/kg to five male and five female CD/Crl:CD rats as an occluded exposure for 24 hours. There were no deaths, no signs of toxicity, no dermal irritation and no observed abnormalities at necropsy.

The dermal LD_{50} of AMPA was greater than 2000 mg/kg (Leuschner, 2002a).

Guinea pigs

The sensitization potential of AMPA (purity 99.2%) was investigated by means of the Magnusson–Kligman Maximization Test in guinea pigs. A group of 20 female Dunkin Hartley guinea pigs were intradermally injected with AMPA at 10% w/v in CMC; 6 days later, 25% w/v in 0.5% CMC was topically applied. Challenge was at a concentration of 25% w/v in CMC.

At challenge, none of the test or control group animals showed a positive response. There was no evidence from the test results that AMPA is a sensitizer in guinea pigs (Cuthbert & Jackson, 1993c).

In a Magnusson–Kligman (maximization test) dermal sensitization study, 10 male Dunkin Hartley guinea pigs were injected with 5% AMPA (purity 98.0%) on day 0, had their application site skin treated with sodium lauryl sulfate on day 6, and then were topically treated with 2 mL of a 50% suspension of AMPA in *aqua ad iniectabilia* on day 7. They were challenged (along with five negative control animals) with 2 mL of a 50% suspension of AMPA in *aqua ad iniectabilia* on day 21. There was no resultant skin irritation in any guinea pig.

The evidence from the test results was that AMPA was a non-sensitizer in this assay (Leuschner, 2002b).

(b) Short-term toxicity studies of AMPA

In a short-term toxicity study, groups of five male and five female Sprague Dawley rats were administered AMPA (purity 99.2%) in CMC at concentrations of 0, 10, 100, 350 or 1000 mg/kg bw per day by oral gavage for 28 days.

There were no treatment-related effects on mortality, clinical signs, body weight, body-weight gains, feed or water consumption or macroscopic findings. There were slight but statistically significant increases in kidney weights in males at 350 and 1000 mg/kg bw per day compared with control group (by 7% and 8%, respectively). Histological examinations revealed a very slight reduction in serous secretion in the mandibular salivary gland of one high-dose male. Whether the minor salivary gland findings is related to treatment is equivocal.

The NOAEL is 100 mg/kg bw per day based on an increase in kidney weights seen at 350 mg/kg bw per day and greater (Heath, Strutt & Iswariah, 1993).

477

In a 90-day toxicity study, groups of 10 male and 10 female Sprague Dawley rats were administered AMPA (purity 99.2%; in CMC) at a concentrations of 0, 10, 100 or 1000 mg/kg bw per day by gavage for 13 weeks. Blood samples were taken from all animals during week 13 for investigation of haematology and clinical chemistry parameters. An ophthalmoscopic examination was undertaken on all animals during pre-trial and on all control and high-dose animals during week 12. All surviving animals were necropsied at termination as were all pre-terminal decedents. Histological examination was carried out on selected tissues from all control and high-dose animals and all pre-terminal decedents and on the kidneys, liver, lungs, submaxillary salivary gland, sublingual salivary gland and parotid salivary gland of all other animals.

There was no treatment-related effect on mortality, clinical signs, body weight, body-weight gain, feed consumption, water consumption, haematology and clinical chemistry parameters, ophthalmoscopic examination, organ weights, macroscopic findings and histological examination. The NOAEL in this 90-day gavage toxicity in rats with AMPA was 1000 mg/kg bw per day (Strutt et al., 1993).

Table 51. Summary of acute toxicity studies of AMPA

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	Reference
Mouse	(Crj:CD-1)	M + F	Oral	99.33	> 5 000	Komura (1996)
Rat	Alpk:APfSD, Wistar	M + F	Oral	100 (assumed)	> 5 000	Leah (1988)
Rat	Sprague Dawley	M + F	Oral	99.2	> 5 000	Cuthbert & Jackson (1993a)
Rat	Sprague Dawley	M + F	Dermal	99.2	> 2 000	Cuthbert & Jackson (1993b)
Rat	CD/Crl:CD	M + F	Dermal	98.0	> 2 000	Leuschner (2002a)
Guinea pig	Dunkin Hartley	F	Sensitization (Magnusson–Kligman Maximization Test)	99.2	Negative	Cuthbert & Jackson (1993c)
Guinea pig	Dunkin Hartley	M	Sensitization (Magnusson–Kligman Maximization Test)	98.0	Negative	Leuschner (2002b)

LD_{50}: median lethal dose

(c) Genotoxicity of AMPA

A much smaller number of studies have been conducted on the glyphosate metabolite, AMPA, as well as the plant metabolites, *N*-acetyl-glyphosate and *N*-acetyl-AMPA. The results are shown in Tables 33, 34 and 35. The in vivo studies (Jensen, 1993c; Kier & Stegeman, 1993; Manas et al., 2009b; see Table 35) investigated the ability of AMPA to induce micronuclei in the bone marrow erythrocytes of mice and have largely been negative although a modest positive response was reported by Manas (2009b) when AMPA was administered by intraperitoneal injection to male mice.

Studies by other investigators using the more relevant oral route of administration did not show an increase in micronuclei in either male or female mice.

In the in vitro studies, increases in mutation in bacteria were not seen for AMPA or the acetylated metabolites. Both positive (Manas et al., 2009b) and negative (Jensen, 1993b,c; Roustan et al., 2014) results were reported in studies of chromosome aberrations and DNA damage for AMPA. AMPA was negative in two studies of unscheduled DNA synthesis in isolated rat hepatocytes (Bakke,

1991; Nesslany, 2002). Studies of chromosome aberrations and gene mutation in mammalian cells using the acetylated metabolites were negative.

(d) Developmental toxicity of AMPA

In a developmental toxicity study, AMPA (purity 99.2%) suspended in CMC was administered to 10 copulated Sprague Dawley female rats per dose by oral gavage at concentrations of 0, 100, 350 or 1000 mg/kg bw per day from days 6 through 16 of gestation. On day 20 of gestation, the dams were terminated, pregnancy status determined and numbers of corpora lutea, implantations and live fetuses recorded. All live fetuses were weighed, sexed and examined for external, visceral and skeletal abnormalities.

There were no mortalities or treatment-related clinical throughout the study. Body-weight gain and feed consumption of the test animals were similar to those of the controls. There were no notable intergroup differences in the incidence of intrauterine deaths or in mean fetal weights. Examination of fetuses for developmental abnormalities and variations of the viscera and skeleton (including state of ossification) showed no intergroup differences.

The NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested (Hazelden, 1992).

2.8 Studies on metabolites: N-acetyl-glyphosate and N-acetyl-AMPA

Metabolism studies in genetically modified soya beans and maize containing the glyphosate-*N*-acetyltransferase gene demonstrated the formation of new metabolites not observed in conventional crops. The major metabolite in the new maize and soya bean varieties was *N*-acetyl-glyphosate (which may be degraded to glyphosate in the rat), whereas glyphosate, *N*-acetyl-AMPA and AMPA were found in low concentrations in the edible parts of the crops. *N*-Acetyl glyphosate and *N*-acetyl-AMPA were reviewed by the JMPR in 2011. The Meeting (2011) concluded that the group ADI of 0–1 mg/kg bw established by the 2004 JMPR for glyphosate and AMPA may also be applied to *N*-acetyl-glyphosate and *N*-acetyl-AMPA as the available toxicological data showed that these plant metabolites have no greater toxicity than the parent glyphosate. The 2004 JMPR decided that an acute reference dose (ARfD) for glyphosate was unnecessary. The 2011 JMPR confirmed that it is not necessary to establish an ARfD for *N*-acetyl-glyphosate or *N*-acetyl-AMPA in view of their low acute toxicity and the absence of any toxicological effects that would be likely to be elicited by a single dose.

(a) Biotransformation of N*-acetyl-glyphosate (company code IN-MCX20)*

A total of 45 male Crl:CD(SD)IGS BR rats were each administered a single oral dose of free acid at 15 mg eq/kg bw of [^{14}C]*N*-acetyl glyphosate (sodium salt; purity 84.3%, radiochemical purity 99.2%) in water. Blood was collected from four animals pre dose and at 0.5, 1, 2, 4, 8, 12, 48 and 72 hours post dose. Excreta were collected from five animals at specified intervals through 168 hours post dose. Plasma, excreta and carcasses were analysed for radioactive content. Selected samples of plasma, urine and faeces were analysed for unchanged parent compound and metabolites.

The mean total recovery was 95.5%, with 66.1% (61.3% within 12 hours of dosage) in urine, 26.4% (25.8% within 48 hours of dosage) in faeces, 2.79% in cage wash and wipe, and 0.23% in residual carcass. More than 90% of the total radioactivity was eliminated 48 hours post dose. C_{max} in blood and plasma were 2.93 and 5.31 µg eq/g at 1 and 2 hours post dose, respectively. Radioactivity was eliminated from blood and plasma with half-life values of 20.1 and 15.6 hours, respectively. Comparison of blood and plasma AUC values indicates that ^{14}C-labelled *N*-acetyl-glyphosate distributed preferentially into plasma.

Unchanged ^{14}C-labelled *N*-acetyl-glyphosate recovered in urine and faeces represented over 99% of the administered radioactivity. A metabolite, glyphosate, was detected in faeces and

represented less than 1% of the total radioactivity. Plasma radioactivity consisted entirely of unchanged ^{14}C-labelled *N*-acetyl-glyphosate (Cheng & Howard, 2004).

(b) Acute toxicity of N-*acetyl-glyphosate and of* N-*acetyl-AMPA*

N-Acetyl glyphosate (purity 84.3% sodium salt, equivalent to 67.4% free acid) was suspended in water and administered to five male and five female Crl:CD(SD) IGS BR fasted (17–20 hours) rats at a dose of 5000 mg/kg bw of free acid, administered as a constant dose volume of 10 mL/kg bw.

One female was found dead at 6 hours after administration, and one female and one male were found dead the following day. Signs of toxicity (seen in all rats) included slight hypoactivity, irregular respiration, liquid faeces, soft faeces, light-brown perineal staining, squinted eyes and brown nasal crust. All survivors were normal 3 days after dosing. Necropsy findings of decedents included mottled or discoloured lungs, discoloured (black) liver, soft stomach, yellow fluid or gel-like clear liquid in stomach, fluid in abdominal cavity, fluid in duodenum, jejunum and ileum.

The LD_{50} of *N*-acetyl glyphosate in rats was greater than 5000 mg/kg bw of free acid (Vegarra, 2004).

N-Acetyl AMPA (purity 97%) suspended in deionized water was administered by oral gavage at a constant dose volume of 20 mL/kg bw at 5000 mg/kg to three Crl (CD)SD female rats.

There were no deaths. Signs of toxicity included diarrhoea, dark eyes, lethargy, high posture, stained fur/skin, wet fur, ataxia and/or hyperreactivity. All the rats had fully recovered 3 days after dosage. All the rats gained weight on days 0–7 and 7–14. There were no dose-related abnormalities at necropsy.

The LD_{50} of *N*-acetyl-AMPA in rats was greater than 5000 mg/kg bw based on the signs of toxicity (Carpenter, 2007).

(c) Subacute toxicity of N-acetyl-glyphosate

Five groups of young adult male and female Crl:CD(SD) rats (10/sex per group) were fed diets containing 0, 180, 900, 4500 or 18 000 ppm *N*-acetyl-glyphosate sodium salt (purity 81.8%) (equal to 0, 11.3, 55.7, 283 and 1157 mg/kg bw per day, respectively, for males and 0, 13.9, 67.8, 360 and 1461 mg/kg bw per day, respectively, for females) for 95 days (males) or 96 days (females).

No adverse effects on body weights or nutritional parameters were observed. The slight decrease in body weight (92% of the control) in the high-dose animals was not considered adverse since statistical significance was not achieved. Statistically significant lower overall mean body-weight gain (86% of control) was observed in males at 18 000 ppm but it was not considered adverse as it was not associated with a statistically significant difference in mean final body weight or in overall mean feed consumption of feed efficiency.

There were neither any treatment-related deaths nor any clinical, ophthalmological or neurobehavioural observations. There were no adverse effects on clinical pathology parameters, organ weights, gross pathology or microscopic pathology in male or female rats. The NOAEL for male and female rats was 18 000 ppm, equivalent to 1157 mg/kg bw per day in males and females, respectively (MacKenzie, 2007).

A supplemental report to the 90-day MacKenzie (2007) study tested dietary disodium *N*-acetyl-*N*-(phosphonomethyl)glycine (purity 63%, expressed as the weight per cent on a free acid basis).

Pooled urine samples for male rat groups I (control), III (180 ppm), V (900 ppm), VII (4500 ppm) and IX (18 000 ppm) were collected on day 82 and for female rats groups II (control), IV (180

ppm), VI (900 ppm), VIII (4500 ppm) and X (18 000 ppm) on day 83 for analysis of IN-MCX20 (*N*-acetyl-glyphosate) and its possible metabolites, IN-B2856 (glyphosate) and IN-EY252 (*N*-acetyl AMPA). On the same days, plasma samples from individual rats were collected for the same analyses.

Concentrations of IN-MCX20 (*N*-acetyl-glyphosate) in the urine increased with the increasing dietary levels of *N*-acetyl-*N*-(phosphonomethyl)glycine. Concentrations of IN-B2856 and IN-EY252 were above the limit of detection at higher dietary levels (900–18 000 ppm) but at or below the limit of detection at 180 ppm. In addition, the concentrations of these metabolites were much higher in urine samples from male rats than from female rats at 4500 and 18 000 ppm. Neither IN-MCX20 nor its metabolites were detected in urine of control rats.

Concentrations of IN-MCX20 (*N*-acetyl-glyphosate) also increased in the plasma samples with increasing dietary levels of *N*-acetyl-*N*-(phosphonomethyl)glycine. Concentrations of IN-MCX20 were less than 1.0 µg/mL for males and females in the 180 ppm dietary group, but increased from a mean of about 2 µg/mL up to about 14.0 µg/mL for the other dietary groups. Little to no IN-B2856 (glyphosate) or IN-EY252 (*N*-acetyl AMPA) was detected in plasma at all dietary levels.

These results confirm that IN-MCX20 (*N*-acetyl-glyphosate) is metabolized in rats to small quantities of IN-B2856 (glyphosate) and IN-EY252 (*N*-acetyl AMPA) (Shen, 2007).

(d) Genotoxicity of N-*acetyl-glyphosate and of* N-*acetyl-AMPA*

A few studies have been conducted on the genotoxicity of the glyphosate metabolite, *N*-acetyl-glyphosate. The results are shown in Tables 36, 37 and 38.

The in vivo studies shown in Table 35 (Murli, 2004; Donner, 2006; Glatt, 2006) that investigated the ability of *N*-acetyl-glyphosate to induce micronuclei in the bone marrow erythrocytes of mice and gene mutations and chromosomal aberrations in CHO cells were negative.

Increases in mutation were also not seen in the in vitro studies.

A smaller number of studies have been conducted on the plant metabolites, *N*-acetyl-AMPA. The results are shown in Tables 33, 34 and 35. The in vivo study (Donner, 2007; Table 35) investigating the ability of *N*-acetyl-AMPA to induce micronuclei in the bone marrow erythrocytes of mice was negative.

In the in vitro studies, increases in mutation in bacteria were not seen; nor were gene mutations in Chinese hamster cells (Glatt, 2007) or chromosomal aberrations in human peripheral blood lymphocytes (Gudi & Rao, 2007).

2.9 Studies on other formulation ingredients

Several publications have reported that glyphosate formulation ingredients and possible contaminants have a greater toxicity than the active ingredient, glyphosate.

Although it was pertinent to consider the toxicity of the known formulants, a detailed review and exhaustive analysis could not be undertaken due to lack of time and confidentiality constraints; producers often consider formulation ingredients proprietary and hence confidential and obtaining this information can be problematic. Nevertheless, based on the reports listed below, it is apparent that some of the formulants may have a greater toxicity than the active ingredient, glyphosate.

Polyethoxylated tallow amine (polyoxyethyleneamine [POEA]; MON 0818; CAS No. 61791-26-2 (tallow); POE n =15)

In a 30-day oral toxicity study, MON 0818 (purity and lot number not reported) was administered to groups of 10 male and 10 female Sprague Dawley rats in the diet at concentrations of

0, 800, 2000 or 5000 ppm (equal to 0, 51.7, 122.8 and 268.7 mg/kg bw per day for males and 0, 63.2, 159.9 and 324.8 mg/kg bw per day for females).

All the treated rats survived until scheduled termination. Soft stools were observed from three high-dose males on four occasions and from eight high-dose females on 23 occasions. Body weight, body-weight gain and feed consumption of high-dose male and female rats were significantly reduced during the study; this was consistent with poor diet palatability. Feed consumption of mid-dose male rats was statistically decreased during the first week of treatment, as was total body weight at the end of the study; however, the final body weight was decreased by only 7% relative to controls. No treatment-related effects were found in mid-dose female rats or in low-dose male and female rats. The absolute and relative organ weights of high-dose male and female rats were decreased consistent with the markedly decreased body weight. Prominent or enlarged lymphoid aggregates in the colon of five high-dose female rats were observed at necropsy.

Because a description of the test material, its lot number, its purity and its concentration, homogeneity and stability in the diet were not provided or determined, an estimate of the dose inducing treatment-related effects on male and female rats cannot be made. In addition, very limited in-life observations and, with the exception of selected organ weights and gross pathology, no post-termination studies or observations were made (Ogrowsky, 1989). As a result, this study was deemed unacceptable and it could not be used to establish a NOAEL or LOAEL.

In a subchronic oral toxicity study of MON 0818 in Sprague Dawley rats, the test material was administered in the diet ad libitum to three groups of 10 male and 10 female rats for 90 days. Target test diet concentrations were 0, 500, 1500 or 4500 ppm (equal to 0, 33.0, 99.3 and 291.6 mg/kg bw per day in males and 0, 39.9, 123.1 and 356.6 mg/kg bw per day in females). A similar, concurrent control group of rats were fed the basal diet only.

Exposure at 1500 and 4500 ppm resulted in statistically and toxicologically significant effects. Toxicity observed at 4500 ppm consists of clinical signs (soft stools, three incidences in two males and 86 incidences in all females) observed from day 16 through day 92 of the study, decreased mean body weights throughout the study (from 12–20% in males and 8–18% in females), and decreased mean total body-weight gains in males (31%) and females (35%). Feed consumption was also significantly reduced throughout most of the study (13 weeks for males and 10 weeks for females), particularly during the first week of the study (32% decrease in males and 27% decrease in females). Since a feed efficiency assessment was not conducted, it is not possible to determine if the decreases in body weights, body-weight gains, and feed consumption were due, in part, to the unpalatability of the diet. Statistically significant changes in haematological parameters observed in females may be a result of the inflammation observed in the intestines. Statistically significant changes in clinical chemistry parameters and organ weights observed in high-dose males and females are likely a result of decreased feed consumption/nutrient absorption and body weight.

At both 1500 and 4500 ppm, microscopic examination conducted at necropsy revealed lesions, including hypertrophy and/or vacuolation of histiocytes in the lamina propria of the ileum in all high-dose males and females, and four mid-dose males and four mid-dose females; hypertrophy and/or vacuolation of histiocytes in the lamina propria of the jejunum in four high-dose males, seven high-dose females and one mid-dose female; sinus histiocytosis in nine high-dose males, six high-dose females and two mid-dose males and females; and accumulation of macrophage aggregates in the cortex and medullary cords of the mesenteric lymph node in eight high-dose males, seven high-dose females and two mid-dose females. These inflammatory changes are likely the cause of the soft stools observed during the study and are considered treatment-related.

No statistically significant treatment-related effects on body weight, body-weight gain, feed consumption, haematological/clinical chemistry parameters and organ weights were observed at the low-dose level of 500 ppm. In addition, no gross abnormalities or histopathological findings related to treatment were observed at this dose level.

Based on treatment-related inflammatory changes at 1500 ppm (equal to 99.3 mg/kg bw per day), the NOAEL for MON 0818 was 500 ppm (equal to 33.0 mg/kg bw per day). The LOAEL was 1500 ppm (equal to 99.3 mg/kg bw per day) based on irritation in the intestines and colon (hypertrophy and vacuolation of histiocytes in the lamina propria of the jejunum and ileum, and histiocytosis and accumulation of macrophage aggregates in the mesenteric lymph node (Stout, 1990).

In a screening study, the potential reproductive toxicity and developmental (prenatal and postnatal) toxicity of MON 0818 (purity 69–73%) was evaluated in CD (Sprague Dawley) rats through two successive generations. The study was designed to evaluate the effects of MON 0818 on male and female reproduction within the scope of a screening study. The study was extended to a two-generation study when a decrease in live litter size was observed at the high-dose level. MON 0818 was administered orally via the diet to three groups of 20 male and 20 female CD rats. Target test diet concentrations were 0, 100, 300 or 1000 ppm (corrected for purity to doses equal to 0, 4.4, 13.4 and 44.5 mg/kg bw per day for males and 0, 9.6, 16.1 and 54.0 mg/kg bw per day for females). A similar concurrent control group of rats were fed the basal diet only. At approximately 10 weeks of age, the F_0 animals were dosed via diet for at least 70 days prior to mating and then to termination (males) or lactation day 21 (females). All F_0 adults were terminated following selection of the F_1 generation on postnatal day 21.

Parents for the F_1 generation were selected from the weaned F_1 litters. Between postnatal day 21 or 22 and 70, the weanling F_1 animals (3 per sex/litter, if possible) were administered the test diet on a mg/kg bw basis (so not to overexpose the rapidly growing F_1 animals) at target concentrations of 0, 6, 18 or 61 mg/kg bw per day for the F_1 males and 0, 7, 22 or 74 mg/kg bw per day for the F_1 females. Beginning on postnatal day 70, the F_1 animals selected for breeding from the control and high-dose groups only (2 per sex/litter) were administered the test diet at a constant concentration (0 or 1000 ppm) for at least 80–88 days prior to mating. The selected F_1 males continued to receive the test diet throughout mating and until termination (after lactation day 4). The selected F_1 females continued to receive the test diet throughout mating, gestation and lactation, until termination (after lactation day 4).

Mortality and clinical signs, body weights, body-weight gains, feed consumption, reproductive function, fertility and mating performance, absolute and relative organ weights, macroscopic abnormalities at necropsy and histopathological findings were recorded for all parental/adult animals. In addition, blood samples for testosterone and/or thyroid hormone concentration determinations were collected from one F_1 male and one F_1 female per litter at the scheduled necropsy. Sperm evaluation (motility and morphology) was also performed on all F_1 male animals at termination. Litter size, viability, clinical signs, body weights, body-weight gains, developmental (sexual and physical) parameters, and macroscopic abnormalities at necropsy were recorded for the F_1 and F_2 pups.

Survival and clinical conditions, mean body weights and feed consumption (pre-mating, gestation, and lactation), reproductive performance, mean organ weights, and macroscopic and microscopic morphology of the F_0 and F_1 parental generations were unaffected at all dose levels. Treatment-related effects were also not seen in estrous cyclicity, spermatogenic end-points and testosterone and thyroid hormone levels of the F_1 generation or in the clinical signs, mean body weights and developmental landmarks of the F_1 and F_2 pups, as well as the litter viability and postnatal survival of the F_2 litters.

Potential treatment-related effects were observed in litter loss, increased mean number of unaccounted-for implantation sites and decreased mean number of pups born, live litter size and postnatal survival from birth to lactation day 4 in the high-dose F_0 females and F_1 litters. These effects were limited to a small number of litters, were not always statistically significant and were not reproduced in the F_2 litters. However, the increased (statistically significant) mean number of unaccounted-for implantation sites exceeded the maximum mean value in the laboratory historical control data. While not statistically significant, the corresponding reduced number of pups born and

live litter size, as well as the reduced postnatal survival, were at or below the limits observed in the laboratory historical control data.

The LOAEL of MON 0818 for reproductive toxicity and offspring toxicity in rats was 1000 ppm (equal to 44.5 mg/kg bw per day) based on litter loss, increase mean number of unaccounted-for implantation sites and decreased mean number of pups born, live litter size and postnatal survival from birth to lactation day 4. The NOAEL for reproductive and offspring toxicity was 300 ppm (equal to 13.4 mg/kg bw per day). The NOAEL for parental systemic toxicity was 1000 ppm (equal to 44.5 mg/kg bw per day). A LOAEL for parental systemic toxicity was not determined (Knapp, 2007).

In a combined repeated-dose toxicity study with the reproduction/developmental toxicity screening test, MON 8109 (coco amine ethoxylates, CAS No. 61791-31-9, (coco); Ave POE $n = 2$); purity 100%) or MON 0818 (purity 100%) was administered to 12 Crl:CD(SD) rats/sex per dose in the diet at dose levels of 0, 30, 100, 300 or 2000 ppm MON 8109 or 1000 ppm MON 0818. The mean compound intake for MON 8109 was 0, 2, 8, 23 and 134 mg/kg bw per day for males and 0, 3, 9, 26 and 148 mg/kg bw per day for females. The mean compound intake for MON 8108 was 0, 2, 8, 23 and 76 mg/kg bw per day for males and 0, 3, 9, 26 and 86 mg/kg bw per day for females. Males were fed the test or basal diets for a total of 71–72 days, and the females were fed the test or basal diets for a total of 69–72 days. Functional observational battery and locomotor activity data were recorded for six males per group near the end of diet administration and for six females per group on lactation day 4. Parental animals were terminated approximately 2.5 weeks after lactation day 4, and offspring were terminated on lactation day 4.

There was no treatment-related mortality. One female in the 1000 ppm MON 0818 group was found dead with dystocia on lactation day 1 and another was euthanized in extremis on gestation day 30 and found to have a ruptured uterus. Increased incidences of red material around the nose, reddened nose and reddened mouth at 2000 ppm MON 8109 in males and females were treatment-related. Mean body-weight losses were noted at 2000 ppm MON 8109 in male and females during the first week of test diet administration. Lower mean body weight and/or body-weight gain with corresponding reduction in feed consumption were usually observed in the animals from this group throughout the study. Absolute and relative organ-weight values that were statistically different from the corresponding control were not treatment related as this difference was due to the significantly lower body weight of the 2000 ppm MON 8109–treated animals. The females from this group had a lower number of implantation sites and lower live litter size. The offspring of these females had lower postnatal survival on postnatal day 0, postnatal day 0–1, postnatal days 1–4 and birth to postnatal day 4 compared to the control group. No effect of treatment was observed in male and female mating and fertility, male copulation and female conception indices, gestation length, functional observational battery, locomotor activity, haematology or serum chemistry. No test-substance–related findings were noted in the 30, 100 or 300 ppm MON 8109 or 1000 ppm MON 0818 group males, females or offspring.

The parental systemic LOAEL was 2000 ppm for MON 8109 (equal to 134 mg/kg bw per day), based on clinical findings and decreased mean body weight, body-weight gain and feed consumption. The parental systemic NOAEL was 300 ppm for MON 8109 (equal to 23 mg/kg bw per day).

The reproductive/developmental LOAEL was 2000 ppm MON 8109 (equal to 134 mg/kg bw per day) based on decreased postnatal survival, lower live litter size on postnatal day 0, lower number of pups born and lower number of implantation sites. The reproductive NOAEL is 300 ppm MON 8109 (equal to 23 mg/kg bw per day).

A parental LOAEL for MON 0818 was not demonstrated in this study. The parental NOAEL was 1000 ppm for MON 0818 (equal to 76 mg/kg bw per day).

The reproductive/developmental LOAEL for MON 0818 was not demonstrated in this study. The reproductive NOAEL was 1000 ppm MON 0818 (equal to 76 mg/kg bw per day) (Knapp, 2008; Nord, 2008).

484

In a developmental toxicity study, MON 0818 (purity 100%) was administered in Mazola Corn Oil to 25 Charles River Crl:CDBr female rats per dose by gavage at dose levels of 0 (corn oil only), 15, 100 or 300 mg/kg bw per day from gestation day 6 through 15. On gestation day 20, all surviving females were terminated for developmental examination. The developmental parameters noted included the number of viable fetuses, early and late resorptions, total implantations and total corpora lutea and the sex and weight of fetuses and external, visceral and skeletal examinations of all fetuses.

Six of the 25 high-dose females died during gestation days 6–15. Clinical signs observed in the high-dose females included rales (12/25), laboured respiration (3/25), yellow uro- (15/25) or anogenital (14/25) matting and mucoid faeces (22/25) compared to none for the control animals. Few to no clinical signs were observed in the mid-dose and low-dose females. High-dose females weighed significantly ($P < 0.01$) less than the controls from study day 9 until termination at study day 20. High-dose females also gained 59% less weight compared to controls during treatment (days 6–16). Body weight was similar to controls in the low- and mid-dose groups. Gravid uterine weight was not affected by treatment in any of the groups. High-dose females ate statistically ($P < 0.01$) less feed compared to the control rats, with the most significant decrease (55% less than controls) on days 6–9 before gradually improvement to comparability with controls by day 16. Overall, the high-dose group ate 29% less than the controls during days 6–16. Feed consumption for the low-dose and mid-dose females was comparable to that of controls throughout the study, except for days 6–9 when the mid-dose group had a statistically significant ($P < 0.05$) decrease. No treatment-related effects were observed on liver weight or gross pathology at necropsy in any of the treated dams.

No treatment-related differences were observed in the mean number of corpora lutea, implantations, live fetuses or resorptions or mean fetal weight. On external examination, the mean number of fetal malformations from the high-dose dams appeared to be high but most were observed in a single fetus and a dose–response relationship was not observed. On visceral examination of the fetuses from the high-dose group, one fetus was missing a urinary bladder, one had stenosis of the right carotid artery and two had situs inversus, but these were not considered treatment related as there was no dose–response relationship for the situs inversus and the others were within the historical control data range. Vertebral anomalies with or without rib anomalies were observed in one fetus in the high-dose group but this was within the range of historical control data. No malformations were observed in the low- or mid-dose groups. Several skeletal variations in the sternebrae and ribs were identified but they were observed in both the control and treated groups at similar incidences and are not considered treatment related.

The maternal toxicity LOAEL for MON 0818 in rats was 300 mg/kg bw per day, based on increased mortality, clinical signs and decreased body weight, body-weight gain and feed consumption. The maternal NOAEL for MON 0818 was 100 mg/kg bw per day.

The developmental toxicity LOAEL for MON 0818 in rats could not be determined as no effects were associated with treatment. The developmental toxicity NOAEL for MON 0818 is 300 mg/kg bw per day (Holson, 2006).

In independent trials of the reverse gene mutation assay in bacteria, strains TA1535, TA1537, TA98 and TA100 of *S. typhimurium* were exposed to MON 0818 (purity not stated). In the first trial, all tester strains were exposed to 0.001, 0.003, 0.01, 0.03 or 0.1 mg/plate with S9 activation and 0.0003, 0.001, 0.003, 0.01 or 0.03 mg/plate without S9 activation. (The S9-fraction was obtained from Aroclor 1254–induced male Sprague Dawley rat liver.) A repeat assay was performed on TA1535 and TA1537 (±S9) using the same concentrations as in in trial one. Because cytotoxicity was not observed with all tester strains, test material concentrations were adjusted for the subsequent mutagenicity trials (trials 3 and 4). Concentrations of MON 0818 from 0.01–1.0 mg/plate with S9 activation and 0.003–0.3 mg/plate without S9 activation were tested in strain TA98; 0.001–0.10 mg/plate with and without

S9 activation in TA100; 0.001–0.1 mg/plate without S9 in TA1535; 0.003–0.3 mg/plate with S9 activation and 0.001–0.1 mg/plate without S9 activation in TA1537.

No evidence of mutagenicity was observed in trial 1. A statistically significant ($P < 0.01$) increase in the number of revertant colonies was observed at 0.03 mg/plate (–S9) in TA98 and 0.0003 mg/plate in TA1535 (–S9); however, the increases were less than twofold and not concentration dependent. When the strains were retested in trials 3 and 4, cytotoxicity was seen at 0.3 mg/plate and above with S9 activation and 0.1 mg/plate and above without S9 activation in TA98; 0.03 mg/plate and above with and without S9 activation in TA100; at 0.1 mg/plate without S9 activation in TA1535; and at 0.1 mg/plate and above with and without S9 activation in TA1537. Although slight increases in the number of revertants were seen at non-cytotoxic concentrations of 0.01 and 0.1 mg/plate with S9 activation in TA98, the increases were less than twofold greater than the solvent controls and did not satisfy the criteria for a positive response. No concentration-dependent increase in the number of revertant colonies was observed in any of tester strains with or without S9 activation.

Overall, no evidence of mutagenicity was observed at non-cytotoxic concentrations with or without S9 activation.

MON 0818 was tested up to cytotoxic concentrations in all strains, but failed to induce a mutagenic response in this test system. The positive controls induced the expected mutagenic responses in the appropriate strain (Stegeman & Li, 1990).

In a bone marrow micronucleus assay, adult male and female ICR(Crl:CD-1) mice (5/sex per dose) were treated once via intraperitoneal injection with 0 or 100 mg/kg MON 0818, which was estimated to be about 61% of the LD_{50} (batch/lot no. PIT-8907-757-I; purity 100%, prepared in corn oil). Bone marrow cells were harvested at 24 and 48 hours following dosing and scored for micronucleated polychromatic erythrocytes. Cyclophosphamide (60 mg/kg) served as the positive control.

No deaths or overt signs of clinical toxicity or cytotoxicity of bone marrow were observed at this dose. Although no toxicity was seen at 100 mg/kg, the selected level was considered acceptable in accordance with the high dose recommended by the USEPA Gene-Tox Program (i.e. when a dose that is not less than 50% of the LD_{50} is used to define the maximum tolerated dose) for the micronucleus assay (Mavournin et al., 1990). Administration of 60 mg/kg cyclophosphamide caused a significant ($P < 0.01$) induction of micronucleated polychromatic erythrocytes in both sexes. There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any harvest time up to the maximum tolerated dose (Stegeman & Kier, 1998).

N,N-bis-(2-hydroxyethyl) alkylamine; synthetic ethoxylated amine, ATMER 163 (CAS No. 70955-14-5; C13-C15; ave POE n =2)

In a 90-day oral gavage toxicity study, ATMER 163 (100% a.i. assumed and batch/lot no. not reported) was administered to 20 Sprague Dawley (Crl:CD[SD]BR) rats per sex per dose at concentrations of 0, 15, 30 or 150 mg/kg bw per day. Deionized water was administered to controls.

Numerous clinical signs were observed in animals at 150 mg/kg bw per day. The most notable signs were wheezing and salivation in all high-dose animals and in some at 30 mg/kg bw per day. Other clinical signs observed in both sexes at 150 mg/kg bw per day included blood crust and/or red discharge (nose), dyspnoea, rhinorrhea, opaque eyes, redness, hunched posture, thinness, urine stains, rough hair, desquamation and an increased incidence of alopecia. Two males at 30 mg/kg bw per day as well as four males and one female at 150 mg/kg bw per day died during the study. Statistically significant body weight and body-weight gain deficits were observed in both sexes at 150 mg/kg bw per day; overall body-weight gains were 30.5% and 15.3% lower than control values in males and females, respectively. Statistically significant decreased feed consumption was seen at 150 mg/kg bw per day in males only. An ophthalmoscopic assessment revealed posterior subcapsular cataracts in males at 30 and 150 mg/kg bw per day and in females at 150 mg/kg bw per day while

complete cataracts were found only at 150 mg/kg bw per day in both sexes. Increased mean values for platelet count, white blood cell count, segmented neutrophil count and lymphocyte count were seen at the 150 mg/kg bw per day dose in both males and females; all of the increases were statistically significant except the increased lymphocyte count in males. These findings are often associated with tissue inflammation which, together with other relevant findings, was observed in the lungs and stomach of both sexes at this dosage. The only noteworthy treatment-related gross pathology findings were in the nonglandular stomach and eyes. The findings in the nonglandular stomach, desquamation and alteration of mucosa, were primarily found in both sexes at 150 mg/kg bw per day, although some alterations of mucosa were also seen in animals at 30 mg/kg bw per day. Opaque eyes, seen in both sexes at 150 mg/kg bw per day, were consistent with the ophthalmoscopic findings of complete cataracts. Treatment-related histopathological findings included inflammation in the lungs of both sexes at 150 mg/kg bw per day and the nonglandular stomach of both sexes at 30 and 150 mg/kg bw per day. The inflammation in lungs might have been due to inadvertent aspiration since previous studies have established that ATMER 163 is a primary irritant. Dose-related incidences of acanthosis in the nonglandular stomach were seen in males and females at 30 and 150 mg/kg bw per day. The only noteworthy finding in the glandular stomach was suppurative inflammation in two females at 150 mg/kg bw per day. In addition, microscopic assessment showed cataracts, mostly bilateral, in the eyes of both sexes at 150 mg/kg bw per day.

There were no toxicologically significant treatment-related effects based on assessment of clinical chemistry and limited assessment of organ weights. Urine analysis was not conducted.

The LOAEL for ATMER 163 in Sprague Dawley rats was 30 mg/kg bw per day based on increased mortality, salivation and posterior subcapsular cataracts in males as well as wheezing and macro- and microscopic changes in the nonglandular stomach of both sexes. The NOAEL is 15 mg/kg bw per day (Zoetis, 1991).

In a subchronic 90-day oral toxicity study, ATMER 163 (purity 100%) was administered via capsule to three groups of four male and four female beagle dogs for 13 weeks at concentrations of 15, 30, or 100 mg/kg bw per day. A similar concurrent control group was given empty capsules.

There were no unscheduled deaths during the study. All the dogs survived until scheduled termination. Exposure at 100 mg/kg bw per day resulted in statistically and toxicologically significant effects. Clinical signs of toxicity included increased incidence of salivation, emesis and soft faeces (noted with mucus alone or mucus and bile-like material). Salivation was observed in all males and females beginning in week 3 of the study (six animals) and continuing over 5–11 weeks. Emesis was also observed in all of the males and females and was first observed during the first 2 weeks of the study in seven animals and continued over 1–11 weeks. Soft mucoid faeces were observed in three males and all the females over 2 to 7 weeks; soft mucoid or bile-particle–containing faeces were observed in the high-dose animals (three males and two females) over 1–3 weeks. All of these clinical signs are considered treatment related based on the high frequency of occurrence and clear dose–response relationship. In addition, mean alanine transaminase levels in females were significantly increased (154%) relative to controls. Microscopic examination at necropsy revealed increased pigment accumulation in the Kupffer cells and bile canaliculi in the livers of all high-dose females. The increased pigment accumulation was not observed in any of the treated males or in the low- and mid-dose females. Other microscopic findings were observed, but were not dose related or were found also found in control animals.

The statistically significant increase (22%) in mean erythrocyte counts observed in high-dose females was within the historical control range. The significant increases (6%) in mean calcium levels observed in the mid- and high-dose females were small, and the observed significant decrease (23%) in mean blood urea nitrogen levels in the mid-dose males did not follow a dose–response pattern. All of the changes are considered incidental to treatment.

No statistically significant effects on body weight, body-weight gain, feed consumption or organ weights were observed at any dose level. In addition no gross abnormalities or ophthalmological changes related to treatment were observed.

The NOAEL for ATMER 163 in rats was 30 mg/kg bw per day based on the clinical signs seen at 100 mg/kg bw per day. The LOAEL was 100 mg/kg bw per day based on clinical signs (increased incidence of salivation, emesis, and soft faeces (with mucus alone or mucus and bile-like material) in males and females, increased alanine transaminase levels in females, and an increased incidence of pigment accumulation in the Kupffer cells and bile canaliculi in the livers of females (Osheroff, 1991).

Armoblen 557 (CAS No. 68213-26-3 (Tallow, POE n = 5/12)

In a four-week oral toxicity study, Armoblen 557 (purity unknown) was administered daily by gavage to groups of five male and five female CD rats at concentrations of 0, 15, 75 or 200 mg/kg bw per day.

All the rats survived until scheduled termination. Salivation in males and females at 75 and 200 mg/kg bw per day was probably due to the taste of the test material and was not considered toxicologically significant. Rales reported in one to three high-dose females were not associated with other effects seen at necropsy and was therefore not considered toxicologically significant. The brown staining around the muzzle occasionally seen in females at 75 mg/kg bw per day and males and females at 200 mg/kg bw per day was also not considered toxicologically significant. Mean body weight was decreased in males (11–17% lower than controls) and females (4–7% lower than controls) at 200 mg/kg bw per day. Overall body-weight gain was decreased in males at 75 mg/kg bw per day (13% lower than controls) and in males and females at 200 mg/kg bw per day (27% and 14% lower than controls, respectively). Overall feed consumption for high-dose females was decreased (10% lower than controls), while it was decreased in high-dose males during week 1 only. Overall feed conversion efficiency was decreased in males at 75 and 200 mg/kg bw per day (13 and 23% lower than controls, respectively).

Changes in haematology and clinical chemistry parameters were either not treatment related or not toxicologically significant. Increases in the absolute and relative adrenal weights in males and females at 200 mg/kg bw per day were not accompanied by microscopic findings and were not considered toxicologically significant.

Based on decreased body weight, body-weight gain and food-conversion efficiency, a LOAEL of 200 mg/kg bw per day and a NOAEL of 75 mg/kg bw per day was established for Armoblen 557 in male CD rats. A LOAEL for Armoblen 557 in female CD rats was not established. The NOAEL in female CD rats was 200 mg/kg bw per day (Higgs, 1994).

MON 59112

In three independent reverse gene mutation assays, *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E. coli* WP2 uvrA were exposed to MON 59112 (assumed 100% purity) in deionized water at concentrations of 0, 1, 3.33, 10, 33.3, 100 or 333 µg/plate with and without S9 activation for the *S. typhimurium* strains and 0, 10, 33.3, 100, 333, 1000 or 3330 µg/plate with and without S9 activation for WP2 uvrA. The S9-fraction was derived from male Sprague Dawley rats induced with Aroclor 1254.

MON 59112 was tested up to cytotoxic concentrations in all strains (≥ 100 µg/plate +S9 and ≥ 33.3 µg/plate –S9 for *S. typhimurium* TA1535, TA1537, TA100 and TA98; ≥ 3330 µg/plate +S9 and ≥1000 µg/plate –S9 for WP2 uvrA) but failed to induce a mutagenic response in this test system. The positive controls induced the expected mutagenic responses in the appropriate strain. There was no evidence of induced mutant colonies over background (Lawlor, 2000).

In a bone marrow micronucleus assay, adult male and female ICR(Crl:CD-1) mice were treated once via oral gavage with MON 59112 (lot no. GLP-9708-8157-I) emulsified in corn oil. Doses of 0, 375, 750 or 1500 mg/kg bw were administered to groups of six male mice and doses of 0, 500, 1000 or 2000 mg/kg bw were administered to groups of six female mice. Bone marrow cells were harvested from the first five survivors at 24 hours (all dose groups) and 48 hours (1500 or 2000 mg/kg bw) following dosing. The harvested bone marrow cells were scored for micronucleated polychromatic erythrocytes and the ratio of polychromatic to normochromatic erythrocytes. Cyclophosphamide (80 mg/kg bw) served as the positive control.

Based on the findings of no substantial differences in the toxicological response of the male or female mice, only the females were administered the limit dose of 2000 mg/kg bw. Two males in the 1500 mg/kg bw and one female in the 2000 mg/kg bw treatment groups died before the scheduled termination. Other toxic signs included hypoactivity, hunched posture, squinted eyes, rough hair coats and faecal stains (1500 mg/kg bw males) and hunched posture and urine stains (2000 mg/kg bw females). There were also significant reductions in the polychromatic to normochromatic erythrocyte ratio for the high-dose males but not the high-dose females. Administration of 80 mg/kg bw cyclophosphamide caused a significant ($P < 0.01$) induction of micronucleated polychromatic erythrocytes in both sexes. There was, however, no significant increase in the frequency of micronucleated polychromatic erythrocytes in any treatment group at either harvest time (Myhr, 2000).

Five glyphosate coformulants were tested for activation of the steroidogenic enzyme aromatase in an in vitro assay (Defarge et al., 2016).

The coformulants tested may have different CAS numbers as the formulations differ. Those tested were (1) pure polyethoxylated tallow amine (POEA; POE-15, CAS No.: 61791-26-2, trade name Emulson AG GPE 3SS) and formulated polyethoxylated tallow amine (POEA/F; CAS No.: 61791-26-2, trade name Emulson AG GPE 3/SSM) form containing 70% of POE-15; (2) alkyl polyglucoside (APG; CAS No.: 383178-66-3/110615-47-9, trade name Plantapon LGC); (3) a mixture of alkyl (C8–10) polyoxyethylene ether phosphates and polyoxyethylene alkyl ether phosphate (POE-APE; CAS Nos.: 68130-47-2 and 50769-39-6, trade name Rolfen Bio); and (4) quaternary ammonium compound (QAC, CAS No.: 66455-29-6, trade name Emulson AG CB 30; and (5) alkyl polyglycoside (CAS No. 110615-49-9, trade name Plantapon LGC).

Aromatase activity was measured by tritiated water release in human JEG3 cells (for discussion on this assay, see Section 2.6f, Table 50). Mitochondrial succinate dehydrogenase activity and membrane integrity were assayed after a 24-hour exposure to assess cytotoxic effects.

The concentrations tested for succinate dehydrogenase activity were derived based on those concentrations reported to be used in glyphosate formulations which can differ according to different formulations: for example, POEA (9 ppm); POEA (18 ppm); APG (800 ppm); POE-APE (100 ppm); and QAC (100 ppm). Statistically significant differences from the controls were determined by a Kruskal–Wallis nonparametric test followed by a post hoc test using significant levels. Aromatase assays were performed at 2.5 ppm of POEA, 120 ppm of APG. The authors report that aromatase activity was decreased by the coformulant alone (POEA, –43%; $P < 0.01$) and slightly by the formulation of the active ingredient plus the coformulant (–25%; $P < 0.05$). Formestane (4-hydroxyandrost-4-ene-3,17-dione), a known aromatase inhibitor, was used as a positive control to demonstrate the specificity of the effect.

1,4-Dioxane

1,4-Dioxane is used primarily as a solvent in the manufacture of chemicals and as a laboratory reagent; it has been noted as being a trace contaminant of glyphosate formulations.

1,4-Dioxane has been classified by the IARC as "possibly carcinogenic to humans (Group 2B)" (IARC, 1987) and, in the National Toxicology Program's fourteenth edition report on carcinogens, as "reasonably anticipated to be a human carcinogen" (NTP, 2016).

Studies in rodents show liver tumours to be consistently reported after chronic oral exposure to 1,4-dioxane. A weight-of-evidence evaluation re-examined mouse liver slides from the 1978 National Cancer Institute bioassay of 1,4-dioxane in drinking water. This re-examination clearly identified dose-related non-neoplastic changes in the liver; specifically, a dose-related increase in the hypertrophic response of hepatocytes, followed by necrosis, inflammation and hyperplastic hepatocellular foci. While 1,4-dioxane does not cause point mutations, DNA repair or initiation, it appears to promote tumours and stimulate DNA synthesis. The weight of the evidence suggests that 1,4-dioxane causes liver tumours in rats and mice through cytotoxicity followed by regenerative hyperplasia. A reference dose (RfD) of 0.05 mg/kg day was proposed to protect against regenerative liver hyperplasia based on a benchmark dose approach (Dourson et al., 2014).

FD&C Blue No. 1

FD&C Blue No. 1 is a blue colourant used in glyphosate formulations. As literature on this compound is sparse, it was run through predictive expert system software (Derek Nexus 5.0.1, Nexus 2.1.0, Lhasa Ltd., Leeds, United Kingdom) in February 2016. The parent compound indicated plausible toxicity with respect to chromosome damage in vitro in mammals due to an alert match with triarylmethane salt and irritation of the eye in mammals due to an alert matched with 4,4'-methylenedianiline.

Availability of supplementary Toxcast/Tox 21 data

In addition to supplementary literature review, Toxcast and Tox21 data searches were conducted on 29 April 2016 for glyphosate coformulants. The Tox21 toolbox (http://ntp.niehs.nih.gov/results/tox21/tbox/index.html) was utilized to access the databases and acquire the data. Data were obtained for two of the glyphosate coformulants: 1,4-dioxane and FD&C Blue No 1. Other typical coformulants were not tested.

In a broad sweep of testing (including AhR, FXR, PPARs, VDR, MMP, p53, NFkB, GR), for FD&C Blue No. 1, the AC50 (µmol/L) results were positive for estrogenic agonist (1 assay only: 1.00E-4) and antagonist activity (1 assay only: 4.79), AR antagonist activity (4.76), aromatase inhibition (1.00E-4), TR antagonism (1.00E-4) and retinoic acid–receptor-related orphan receptor antagonism (1.00E-4). For 1,4-dioxane, the AC50 (µmol/L) results were negative across all assays.

3. Observations in humans

3.1 Occupational exposure: Biomonitoring studies

Occupational exposure to glyphosate can occur via dermal and inhalation routes. However, in vitro and in vivo percutaneous absorption studies suggest that dermal penetration of glyphosate formulation is very limited and that exposure through inhalation is minimal due to the low vapour pressure of glyphosate.

Both passive dosimetry and biomonitoring have been used as techniques to assess exposure. Biomonitoring results represent systemic (internal) exposure, whereas passive dosimetry results quantify external deposition. There is general agreement that biological measurements obtained through biomonitoring provide the most relevant information for safety assessments (Franklin, Muir & Moody, 1986; Chester & Hart, 1986).

The Farm Family Exposure Study was a biomonitoring study supported by seven agricultural companies. In this study, eligible farm families from Minnesota and South Carolina were randomly

selected from a roster of licensed private pesticide applicators. Participant families consisted of a farmer, their spouse and at least one child between the ages of 4 and 17 years; lived on the farm; and planned to apply one of the target pesticides (glyphosate, chlorpyrifos, 2,4-D) to at least 10 acres (4.1 hectares) of land within 1 mile (1.6 kilometres) of their house. For each family member, geometric means were calculated for 24-hour composite urinary samples, with a 1 ppb limit of detection, the day before, the day of and for 3 days after the pesticide application. For the farmers, the peak geometric mean concentrations were 3 ppb for glyphosate, 64 ppb for 2,4-D and 19 ppb for the primary chlorpyrifos metabolite. For the spouses and children, the percentage with detectable values varied by chemical, although the average values for each chemical did not vary during the study period. The applicators had the highest urine pesticide concentrations, children had much lower values and spouses had the lowest values. Exposure to family members was largely, though not exclusively, determined by the degree of direct contact with the application process. The exposure profile varied for the three chemicals for each family member (Mandel et al., 2005).

As part of the Farm Family Exposure Study, urinary glyphosate concentrations were evaluated for 48 farmers, their spouses and their 79 children (4–18 years of age). The study authors stated that they evaluated 24-hour composite urine samples for each family member the day before, the day of and for 3 days after a glyphosate application. On the day of application, 60% of the farmers had detectable levels of glyphosate in their urine on the day of application. The geometric mean concentration was 3 ppb, the maximum value was 233 ppb, and the highest estimated systemic dose was 0.004 mg/kg. Those farmers who did not use rubber gloves had higher geometric mean urinary concentrations than the other farmers (10 ppb vs 2.0 ppb). For spouses, 4% had detectable levels in their urine on the day of application; their maximum value was 3 ppb. For children, 12% had detectable glyphosate in their urine on the day of application, with a maximum concentration of 29 ppb. All but one of the children with detectable concentrations had helped with the application or were present during herbicide mixing, loading or application. None of the systemic doses estimated in this study approached the USEPA reference dose for glyphosate of 2 mg/kg bw per day (Acquavella et al., 2004).

Some earlier biomonitoring studies were performed on silvicultural workers who sprayed a glyphosate formulation in a variety of forestry and tree farming activities. In one study, the United States Department of Agriculture's Forest Service, in collaboration with Monsanto and the University of Arkansas, sponsored a study to investigate exposure to glyphosate of workers at two forestry nurseries (Phipps Nursery in Oregon and Ashe Nursery in Massachusetts) where glyphosate was used for weed control. Urine samples were collected from the weeders and scouts prior to working with glyphosate and for an eight-month period thereafter. Continuous total urine sampling was conducted for the first 12 consecutive weeks of the study, after which a 24-hour sample was collected each Wednesday for the next five months.

Of the 355 daily urine samples analysed, none were found to contain quantifiable levels of glyphosate. The limit of quantification was 10 ppb (Lavy et al., 1992).

A separate collaborative study conducted by the United States Department of Agriculture (USDA) Forestry Service, Georgia Tech Research Institute and Monsanto examined the effects of exposure to glyphosate on applicators using a hand-held directed spray foliar application at three sites maintained by the USDA Forestry Service. Urinary samples were collected for 5 days after exposure. Of the 96 urine samples analysed, five were found to contain quantifiable levels of glyphosate. The highest glyphosate measure was 14 ppb and the highest estimated internal dose was 0.0006 mg/kg body weight (Cowell & Steinmetz 1990).

Two other studies have been conducted to measure exposure of forestry workers to glyphosate during normal silvicultural applications. In the Finnish study (Jauhiainen et al., 1991), urine samples were collected at the end of each day from workers spraying glyphosate for 5 consecutive days in August 1988. In addition, each worker had an ECG; underwent haematology, clinical chemistry and pulmonary function tests and a general clinical examination (including blood pressure, pulse rate and pressure craft of hands); and was interviewed for a health questionnaire on the first day and last day. All urine samples had less than detectable concentrations of glyphosate. There were no statistically significant differences in the findings of the medical examinations conducted before and after exposure (Jauhiainen et al., 1991).

The Canadian study of forestry workers following normal silviculture uses of glyphosate was conducted over two growing seasons (in 1986) and involved 45 workers conducting various operations. Glyphosate was not detected in the majority of urine samples. For the two flagmen and the operator, glyphosate concentrations in all urine samples were less than 0.03 ppm (the limit of quantitation). In contrast, 14 of 33 urine samples from the mixer and two urine samples for the foreman contained glyphosate concentrations greater than 0.03 ppm. Maximum glyphosate concentrations in the foreman's and mixer's urine were 0.043 and 0.055 ppm, respectively. In the follow-up study in 1987, glyphosate concentrations in urine of exposed workers were very low. In the majority of samples, glyphosate was not detectable. In those samples with detectable levels of glyphosate, concentrations were less than 0.1 ppm in all cases and typically less than 0.035 ppm (Centre de Toxicologie du Quebec, 1988).

3.2 Occupational exposure: Epidemiological studies with specific reference to cancer outcomes

The pre-agreed evaluation process and Tier 1 screening criteria used to evaluate epidemiological studies on malathion (and diazinon and glyphosate) are described in "Section 2.2: Methods for the evaluation of epidemiological evidence for risk assessment" of the Meeting report[9].

Identification of compound/cancer sites and screening of papers

This assessment was limited to studies of cancer outcomes; numerous studies have assessed risks for neurodevelopmental, neurodegenerative and reproductive outcomes, among other health outcomes. Restricting the assessment to cancer outcomes was partly driven by reasons of feasibility: a clinically relevant adverse effect size (or an acceptable level of risk) for a non-cancer outcome must be defined, and the methodologies for hazard identification and characterization based on observational epidemiological findings of non-carcinogenic adverse effects are less well-established than those for cancer (Clewell & Crump, 2005; Nachman et al., 2011).

The pre-agreed evaluation process and Tier 1 screening criteria used to evaluate epidemiological studies on glyphosate (and malathion and diazinon) are described in "Section 2.2: Methods for the evaluation of epidemiological evidence for risk assessment" of the Meeting report[10].

The IARC monographs on glyphosate, malathion and diazinon refer to a total of 45 epidemiological studies. Two studies published since the IARC monographs, which evaluated at least one of malathion, diazinon or glyphosate in relation to cancer outcomes, were also identified (Lerro et al., 2015; Koutros et al., 2015).

[9] Pesticide residues in food 2016: Special session of the joint FAO/WHO meeting on pesticide residues May 2016: Report 2016 (http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/).

[10] Pesticide Residues in Food 2016: Special session of the joint FAO/WHO meeting on pesticide residues May 2016: Report 2016 (http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/).

The 45 publications referred to in the IARC monographs and the two publications since (Lerro et al., 2015; Koutros et al., 2015) covered a total of 48 compound/cancer site combinations. The current evaluation focuses on the six compound/cancer site combinations for which IARC identified positive associations from the body of epidemiological evidence, that is, those associations noted in Section 6.1 of the monographs, and which underpin the IARC's evaluation of "limited evidence" in humans for the carcinogenicity of malathion, diazinon and glyphosate. The definition for limited evidence of carcinogenicity used by the IARC is as follows: "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence" (IARC, 2015).

The compound/cancer site combination for glyphosate was NHL. The evaluation of the relevant publications is summarized in Table 52.

During the identification of relevant publications, stand-alone analyses for specific subtypes of NHL (of which there are many subtypes) were noted. The risk was not evaluated separately for subtypes of NHL as there was insufficient evidence (too few studies or small numbers of cases), or for other haematopoietic and lymphoid tumours as the positive associations identified by the IARC were for total NHL.

Overview of studies included in evaluation

The IARC monograph on malathion (IARC, 2015) already provides a good overview of the epidemiological studies which have assessed pesticide exposures and cancer risk. Therefore, only a brief summary (largely based on the IARC monograph) of the studies contributing to the current evaluation is provided here to give context.

The Agricultural Health Study (AHS) is a prospective cohort study of pesticide applicators (predominantly farmers; $n \approx 52\,000$) and their spouses ($n \approx 32\,000$) from Iowa and North Carolina, United States of America, enrolled in 1993–1997. The AHS has examined a range of cancer outcomes, and published updated analyses with longer periods of follow-up (e.g. Beane Freeman et al., 2005; De Roos et al., 2005; Koutros et al., 2013; Alavanja et al., 2014; Jones et al., 2015; Lerro et al., 2015). Information on participants' use of 50 pesticides and other determinants of exposure was collected retrospectively via baseline and two follow-up questionnaires. Cumulative lifetime exposure estimates were calculated. Validation studies have been conducted to assess the reliability and accuracy of exposure intensity scores (a component of the exposure assessment) (Coble et al., 2005; Hines et al., 2008; Thomas et al., 2010). The impact of exposure misclassification in this study was to bias risk estimates towards null (Blair et al., 2011).

The United States Midwest case–control studies are three population-based case–control studies of cancer conducted in Nebraska (Zahm et al., 1990), Iowa and Minnesota (Brown et al., 1990; Cantor et al., 1992), Kansas (Hoar et al., 1986), which have subsequently been pooled (748 cases/2236 controls) for analysis of NHL in white males only (Waddell et al., 2001; De Roos et al., 2003; Lee et al., 2004). Information on participants' occupational use of pesticides was collected retrospectively via questionnaire. There were some differences in case ascertainment and exposure assessment methods between the three studies. For 39% of the pooled study population, proxy respondents were used (Waddell et al., 2001), for whom recall of specific pesticide use could be problematic and subject to recall bias which may differ for cases and controls. De Roos et al. (2003) (same study population as Waddell et al., 2001) performed an extensive evaluation and adjustment for other pesticides.

The Cross-Canada Case–control Study of Pesticides and Health is a population-based case–control study of haematopoietic cancers in men diagnosed during 1991–1994 across six Canadian provinces (McDuffie et al., 2001). It includes 517 NHL cases and 1506 controls. A questionnaire was administered by post, followed by a telephone interview for those who reported pesticide exposure of 10 hours/year or more, and for a 15% random sample of the remainder. The study was not restricted to pesticide exposure experienced by a specific occupational group (McDuffie et al., 2001). Further

analyses stratified by asthma/allergy status – to assess possible effect modification by immune system modulation – have been conducted (Pahwa et al., 2012). The study has a large sample size and detailed information of pesticide exposures; however, the proportion exposed to pesticides was low.

These three sets of studies were deemed as high quality and highly informative by the IARC Working Group (IARC, 2015).

A number of other case–control studies of pesticide exposure and cancer risk were included in this evaluation: the Florida Pest Control Worker study (Pesatori et al., 1994); nested case–control studies within the United Farm Workers of America cohort study (Mills, Yang & Riordan, 2005); a population-based case–control study of prostate cancer in British Columbia, Canada (Band et al., 2011); and case–control studies of NHL/haematopoietic cancers from Sweden (Hardell et al., 2002, Eriksson et al., 2008), and France (Orsi et al., 2009). The IARC Working Group (IARC, 2015) noted substantial limitations in these studies, either in relation to exposure assessment, scope for and variation in exposure misclassification, lack of detail reported in publication which hindered interpretation, lack of specificity due to high correlations between use of different pesticides, and limited power.

Strengths and limitations of studies included in evaluation

The included studies predominantly examined the occupational pesticide exposures of farmers and other pesticide applicators, with the vast majority of research being on males only. None of the studies assessed exposure via food consumption or ambient exposure from agriculture (e.g. spray drift). The scientific evidence available is therefore limited in its generalizability and the extent to which it can be translated to general population exposure scenarios and levels that would be associated with pesticide residues. Nonetheless, these observational epidemiological studies provide insight into real-world exposure scenarios, and allow for observation of the species of interest (humans) over long follow-up time periods relevant to cancer.

The number of high quality studies is relatively small. Typically the number of exposed cases in studies is small, particularly when evaluating specific pesticides, which limits study power.

Relatively few studies have assessed exposure quantitatively, meaning the epidemiological evidence available to inform/establish dose–response relationships is very limited. Exposure misclassification is a potential issue for all studies. This is expected to be largely non-differential for cohort studies (i.e. the AHS), resulting in attenuation of risk estimates. All except one of the studies included are case–control studies, and these may be affected by recall bias, that is, cases and controls recall past pesticide exposure with differing accuracy, leading to differential exposure misclassification which can bias risk estimates either towards or away from the null. As a cohort study, the AHS avoids recall bias.

Given that studies focused on occupational exposures among farmers/pesticide applicators, it is unlikely that they were exposed to only one specific pesticide. As a result, confounding, possible effect modification and additive/multiplicative effects due to co-exposures are all concerns. However, many studies were able to adjust risk estimates for other pesticide co-exposures, which yields more accurate risk estimates.

There are some issues in terms of comparing studies and evaluating the consistency of evidence overall. Results of studies may appear heterogeneous, but usually there are too few studies to really assess consistency and heterogeneity. Exposure assessment methods and referent groups vary between studies.

Finally, changes in disease classifications (particularly NHL) or screening/diagnosis rates (prostate cancer) over time may limit comparability between studies.

494

Publication bias

A formal analysis of publication bias was not undertaken because the number of studies (risk estimates from non-overlapping study populations) available were few and funnel plot tests for asymmetry should be used only where there are at least 10 studies because otherwise statistical power is insufficient to distinguish true asymmetry from chance (Higgins & Green, 2011; Sterne et al., 2011). Other formal objective statistical tests require an even larger number of studies, typically at least 30, to achieve sufficient statistical power (Lau et al., 2006). As a result, publication bias cannot be fully excluded. However, given the very considerable resources invested in these types of (large, difficult exposure assessment) studies, it is unlikely that results would go unpublished.

Summary of evidence for an association between glyphosate and NHL

This evaluation considered several aspects of each study and of all the studies combined, including factors which decrease the level of confidence in the body of evidence, including risk of bias, unexplained inconsistency, and imprecision, and factors which increase the level of confidence, including large magnitude of effect, a dose–response relationship, residual confounding and consistency (Guyatt et al., 2008; Morgan et al., 2016).

The risk estimates findings for each study are summarized in Table 52, and findings for non-quantitative exposure assessment (predominantly ever- vs never-use) are shown in the forest plot below.

Table 52. Results of Tier 1 evaluation and summary of publications by glyphosate/cancer site

Study/ Location	Glyphosate / NHL	Reference
Meta-analysis	Qualitative exposure only – ever-/never-use of glyphosate Meta risk ratio: 1.5 (95% CI: 1.1–2.0) Meta-analysis includes McDuffie et al. (2001); Hardell et al. (2002); De Roos et al. (2003, 2005a); Eriksson et al. (2008); and Orsi et al. (2009). *Ns* for each meta-analysis not presented	Schinasi & Leon (2014)
Agricultural Health Study	Quantitative exposure (cumulative exposure days; intensity-weighted cumulative exposure days [years of use × days/year × estimated intensity level]; in tertiles) Risk estimates – aRR (95% CI) Ever-use 1.1 (0.7–1.9) LED 1–20.0 1.0 (ref.) LED 21–56 0.7 (0.4–1.4) LED 57–2678 0.9 (0.5–1.6) *P* for trend 0.73 IW-LED 0.1–79.5 1.0 (ref.) IW-LED 79.6–337.1 0.6 (0.3–1.1) IW-LED 337.2–18241 0.8 (0.5–1.4) *P* for trend = 0.99 Total *N* = 54 315 (49 211/36 823, depending on the analysis), with 92 incident NHL cases (for ever-use; and 61 for analysis based on tertiles of exposure)	De Roos et al. (2005)
United States Midwest case–control studies	The study population overlaps with that of De Roos et al. (2003). See comment below Qualitative – ever/never (analysis stratified by asthmatics vs non asthmatics) Risk estimates – aRR (95% CI) Non-asthmatics: 1.4 (0.98–2.1) Asthmatics: 1.2 (0.4–3.3) Total *N* = 3208 (872 NHL cases, 2336 controls). *N* = 53/91 glyphosate-exposed NHL cases/controls for non-asthmatics and 6/12 glyphosate-exposed NHL cases/controls for asthmatics	Lee et al. (2004)

Study/ Location	Glyphosate / NHL	Reference
	The study population overlaps with Lee et al. (2004) and total *N* is smaller, but as an exception this study was not excluded in the assessment of consistency of risk estimates as it provides overall risk estimates which are comparable with other studies, while Lee et al. (2004) only provides risk estimates stratified by asthma diagnosis Qualitative (ever/never) Risk estimates – aOR (95% CI) From a logistic regression model: Exposed 2.1 (1.1–4.0) From the hierarchical regression model: Exposed 1.6 (0.9–2.8) Both adjusted for other pesticides Total *N* =2 583 (650 NHL cases, 1 933 controls). *N* = 36 exposed cases; *N* = 61 controls	De Roos et al. (2003)
	Excluded – as this study is pooled in De Roos et al. (2003) and Lee et al. (2004) Qualitative exposure only – ever-/never-use of glyphosate Risk estimates – OR (95% CI) Ever-use = 1.1 (0.7–1.9) Total *N* = 1867 (622 cases, 1245 controls) *N* = 26 exposed cases	Cantor et al. (1992)
Cross-Canada Study of Pesticides and Health	Quantitative exposure – days of use per year (3 categories – cutpoints are given). Risk estimates – OR (95% CI) Ever-use: 1.2 (0.83–1.74) Unexposed 1.0 (ref.) >0–<=2 days/year 1.0 (0.63–1.57) > 2 days/year 2.12 (1.20–3.73) *P* trend = NR Total *N* = 2 023 517 cases, 1 506 controls (overall) *N* = 51 exposed cases, 133 exposed controls	McDuffie et al. (2001)
Sweden – note that there is some overlap between Eriksson et al. (2008), Hardell et al. (2002) and Hardell & Eriksson (1999)	Quantitative exposure – days of use per year (2 categories – cutpoints are given). Risk estimates – aOR (95% CI) Ever-use: 2.02 (1.10–3.71) Risk estimates – aOR (95% CI) Non-farmers: 1.0 (ref.) ≤ 10 days/year: 1.69 (0.7–4.07) > 10 days/year: 2.36 (1.04–5.37) *P* trend = NR Total N = 1926 (910 cases, 1016 controls) *N* = 29 exposed cases; *N* = 18 exposed controls	Eriksson et al. (2008)
	Qualitative exposure only – ever-/never-use of glyphosate. A pooled analysis of Nordström et al. (1998) (NHL subtype only, not evaluated separately here) and Hardell & Eriksson (1999) Risk estimates – aOR (95% CI) Ever-use: 1.85 (0.55–6.20) Total *N* = 1 656 (515 cases, 1 141 controls) *N* = 8 exposed cases; *N* = 8 exposed controls.	Hardell et al. (2002)
	Exclude as this study is pooled in Hardell et al. (2002). Qualitative exposure only – ever-/never-use of glyphosate	Hardell & Eriksson (1999)

Study/ Location	Glyphosate / NHL	Reference
France	Qualitative – ever-/never-use of glyphosate Risk estimates – aOR (95% CI) Ever-use: 1.0 (0.5–2.2) N = 12 exposed cases; N = 24 exposed controls (The researchers report evaluating quantitative duration with respect to median duration of exposure among exposed controls as never exposed; duration < median; duration > median, but neither the median cutpoint nor ORs/test for trend results are presented in the paper, so this study cannot contribute any information for quantitative risk assessment.)	Orsi et al. (2009)

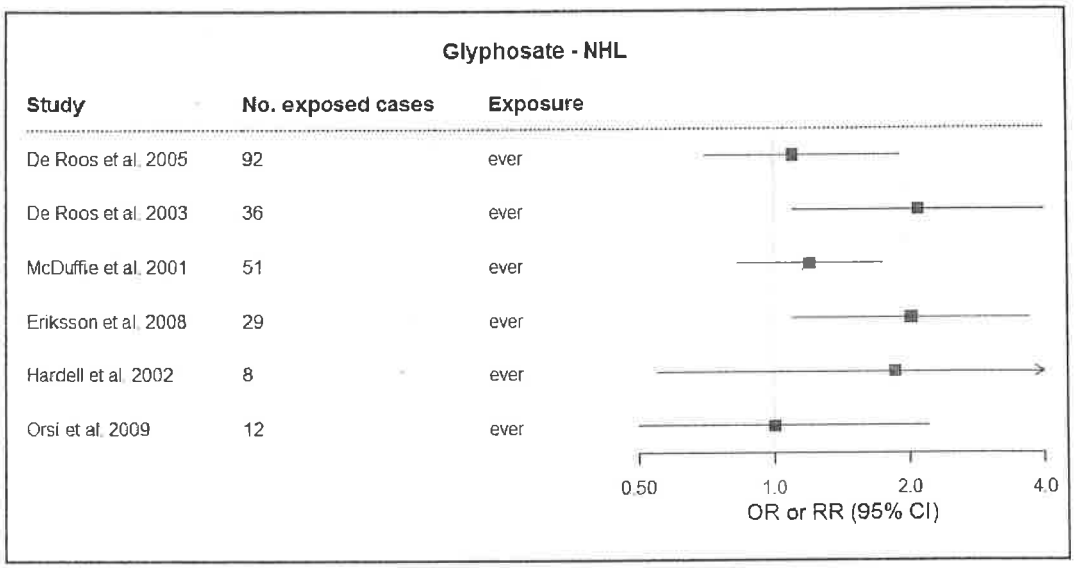
aOR: adjusted odds ratio; aRR: adjusted risk ratio; CI: confidence interval; IW-LED: intensity-weighted lifetime exposure days, defined as number of years of use × number of days used per year × personal protective equipment use reduction factor × intensity level score (a unit-less score which reflects a combination of self-reported pesticide exposure modifiers, e.g. pesticide mixing status, application method, equipment repair activities); LED: lifetime exposure days, defined as number of years of use × number of days used per year; NHL: non-Hodgkin lymphoma; N: sample size; NR: not reported; OR: odds ratio; ref.: reference

The maximally adjusted risk estimates were extracted.

The Glyphosate / NHL evaluation included seven studies (McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Lee et al., 2004; De Roos et al., 2005; Eriksson et al., 2008; Orsi et al., 2009) and one meta-analysis (Schinasi & Leon, 2014). Three studies used quantitative exposure metrics, although, the units differed: lifetime exposure days and intensity-weighted lifetime exposure days (De Roos et al., 2005) and days of use per year (McDuffie et al., 2001; Eriksson et al., 2008). The AHS found no evidence of elevated risk of NHL or exposure–response associated with glyphosate exposure (De Roos et al., 2005). Elevated risks were reported in various case–control studies. De Roos et al. (2003) reported significant elevated risk of NHL associated with ever- versus never-use of glyphosate (OR: 2.1 [1.1–4.0] and a borderline nonsignificant OR (1.6 [0.9–2.8]) with an alternative Bayesian hierarchical model) from the United States Midwest pooled case–control studies. There was no evidence of effect modification by asthma diagnosis in the United States Midwest pooled case–control studies (Lee et al., 2004). Ever-use of glyphosate was not associated with risk of NHL in the Cross-Canada Case–control Study of Pesticides and Health, but using glyphosate for longer than 2 days per year was associated with a significant elevated risk (OR: 2.12; 95% CI: 1.20–3.73), although there was no indication of an exposure–response relationship across exposure categories (McDuffie et al., 2001). Eriksson et al. (2008) reported significant elevated risk of NHL associated with ever-use (OR: 2.02 [1.10–3.71]) and use of glyphosate for longer than 10 days per year (OR: 2.36 [1.04–5.37]) and indicate an exposure–response relationship. A pooled study of two Swedish case–control studies reported a nonsignificant elevated risk of NHL for ever-use of (OR: 1.85 [0.55–6.2]); however, with only eight exposed cases, this study had limited power to detect associations (Hardell et al., 2002). Orsi et al. (2009) found no evidence of association. Schinasi & Leon (2014) reported a meta risk ratio of 1.5 (95% CI: 1.1–2.0) for ever- versus never-use of glyphosate. The meta-analysis included the AHS (De Roos et al., 2005) and five out of the six case–control studies included in this evaluation (McDuffie et al., 2001; Hardell et al., 2002; De Roos et al., 2003; Eriksson et al., 2008; Orsi et al., 2009).

497

Fig. 3. Forest plot for risk estimates of NHL associated with glyphosate, from studies with qualitative exposure categories



Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta-analysis. However, it is notable that the AHS, which is the only cohort study and is large and of high quality, found no evidence of association at any exposure level.

Comments

Biochemical aspects

In studies with radiolabelled glyphosate in rats, glyphosate was rapidly absorbed from the gastrointestinal tract following oral intake, but only to a limited extent (about 20–30%) (McEwen, 1995). Elimination was fast and virtually complete within 72–168 hours, with the majority being excreted during the first 48 hours (McEwen, 1995). Most of the excretion occurred in faeces, largely as unabsorbed dose, and in the urine. Biliary excretion of glyphosate was negligible. Less than 1% of the administered dose was retained in tissues 168 hours post-administration. Highest residues were detected in bone, followed by kidney and liver (Powles, 1992b; Ridley & Mirly, 1988). This pattern of absorption, distribution and elimination was independent of dose, treatment regimen and sex of the test animals. Peak plasma concentrations of radiolabel were observed at 6 and 2 hours after administration in male and female rats, respectively (McEwen, 1995). The estimated half-life for whole-body elimination of the radiolabel was about 5.9–8.3 hours (McEwen, 1995).

There was very little biotransformation of glyphosate; the only metabolite, AMPA, accounted for 0.2–0.7% of the administered dose in excreta; the rest was unchanged glyphosate (Macpherson, 1996).

Toxicological data

Glyphosate has low acute oral toxicity in mice (LD_{50} > 2000 to > 10 000 mg/kg bw; no lethality at 2000 mg/kg bw) (Shirasu & Takahashi, 1975)and rats (LD_{50} 5600 mg/kg bw) (Heenehan, 1979a), low acute dermal toxicity in rats (LD_{50} > 2000 mg/kg bw) (Cuthbert & Jackson, 1989b; Komura, 1995c; Doyle, 1996b; Talvioja, 2007b; Do Amaral Guimaraes, 2008b; Haferkorn, 2009b, 2010c,d; Simon, 2009b) and rabbits (LD_{50} > 5000 mg/kg bw) (Heenehan, 1979b; Blaszcak, 1988b;

Reagan, 1988a), and low acute inhalation toxicity in rats (LC_{50} > 5.48 mg/L). Glyphosate was not irritating to the skin of rabbits (Heenehan, 1979c; Reagan & Laveglia, 1988b; Hideo, 1995a; Doyle, 1996c; Arcelin, 2007c; Talvioja, 2007c; Canabrava Frossard de Faria, 2008a; You, 2009c; Leuschner, 2009a,c, 2010a). Glyphosate produced moderate to severe eye irritation in rabbits, with irreversible corneal opacity in one study as a consequence of the low pH of the test material in solution (Arcelin, 2007d; Blaszcak, 1988d; Hideo, 1995b; Johnson, 1997; Merkel, 2005e; Talvioja, 2007d; You, 2009d). Glyphosate was not sensitizing in guinea pigs (Doyle, 1996d; Haferkorn, 2009d, 2010f,g; Hideo, 1995c; Lima Dallago, 2008; Merkel, 2005f; Richeux, 2006; Talvioja, 2007e; Simon, 2009d; You, 2009e) or mice (Betts, 2007; Török-Bathó, 2011) as determined by the Magnusson–Kligman maximization test, the Buehler test and the local lymph node assay.

In short-term studies of toxicity in different species, the most notable effects were clinical signs related to gastrointestinal irritation, decreased body weight, salivary gland changes (hypertrophy and increase in basophilia of cytoplasm of acinar cells), histological findings in the caecum and hepatotoxicity.

In short-term studies in mice, reduced body weight was seen at a dietary concentration of 50 000 ppm (equal to 9710 mg/kg bw per day) (Tierney & Rinehart, 1979). The NOAEL for decreased body weight was 10 000 ppm (equal to 1221 mg/kg bw per day) (Kuwahara, 1995). Effects on the salivary glands were observed in mice in only one study out of four, at 6250 ppm (equal to 1065 mg/kg bw per day) (Chan & Mahler, 1992). The NOAEL for the salivary gland effects in mice was 3125 ppm (equal to 507 mg/kg bw per day) (Chan & Mahler, 1992). The overall NOAEL in short-term studies in mice was 3125 ppm (equal to 507 mg/kg bw per day), and the overall LOAEL was 6250 ppm (equal to 1065 mg/kg bw per day).

In 90-day toxicity studies in rats, common findings included soft faeces, diarrhoea, reduced body-weight gain and decreased food utilization at dietary concentrations of 20 000 ppm (equal to 1262.1 mg/kg bw per day) and above. The lowest NOAEL was 371.9 mg/kg bw per day. A decrease in urine pH was frequently noted owing to the acidic nature of the compound and excretion as glyphosate in the urine. In two 90-day dietary toxicity studies, an increase in caecum weight (at 10 000 ppm, equal to 569 mg/kg bw per day) and histological findings in the caecum (at 50 000 ppm, equal to 3706 mg/kg bw per day) (Kinoshita, 1995; Coles et al., 1996) were observed. In rats, effects on the salivary gland were seen in two out of seven 90-day studies starting at 12 500 ppm (equal to 811 mg/kg bw per day). The NOAELs for effects on the salivary gland were 300 and 410 mg/kg bw per day. The overall NOAEL in short-term studies in rats was 300 mg/kg bw per day, and the overall LOAEL was 10 000 ppm (equal to 569 mg/kg bw per day).

In four 90-day toxicity studies in dogs, the most notable effects were loose stools, decreased body weight and reduced feed consumption (Hodge, 1996; Yoshida, 1996; Prakash, 1999; Gaou, 2007). In one study, there were no treatment-related effects at doses up to 40 000 ppm (equal to 1015 mg/kg bw per day) (Yoshida, 1996). The lowest NOAEL and LOAEL were 300 mg/kg bw per day and 1000 mg/kg bw per day, respectively.

Seven 1-year toxicity studies in dogs are available. In one study, changes in faeces were observed at 100 mg/kg bw per day and above. The NOAEL was 30 mg/kg bw per day (Teramoto, 1998). However, these results were not reproduced in four other studies with administration via capsules at 300 or 500 mg/kg bw per day (Reyna & Ruecker, 1985; Goburdhun, 1991; Haag, 2008). In the remaining six studies, the NOAELs ranged from 8000 ppm (equal to 182 mg/kg bw per day; Nakashima, 1997) to 500 mg/kg bw per day (Reyna, 1985; Haag, 2008), and the LOAELs ranged from 30 000 ppm (equal to 926 mg/kg bw per day; Brammer, 1996) to 1000 mg/kg bw per day (Goburdhun, 1991).

The overall NOAEL in the 90-day and 1-year toxicity studies in dogs was 15 000 ppm (equal to 448 mg/kg bw per day), and the overall LOAEL was 30 000 ppm (equal to 926 mg/kg bw per day).

The Meeting compiled the tumour incidence data for all relevant mouse and rat studies in order to undertake statistical analysis and investigate any potential pattern of occurrence across studies. In addition, incidences of tumours of lymphatic tissues were summarized, as these were

identified as possible targets of relevance from the review of epidemiological cancer studies. However, the Meeting recognized that the relationship between tumours of lymphatic tissues in rodents and humans has not been clearly established.

Nine carcinogenicity studies in mice were available. Two studies were considered to be of insufficient quality to be included in the assessment (Bhide, 1988; Vereczkey & Csanyi, 1982, revised 1992). Effects such as loose stools, reduced body weights and decreased feed consumption were noted in most of the studies (Pavkov & Turnier, 1987; Atkinson et al., 1993a; Sugimoto, 1997; Takahashi, 1999a). The overall NOAEL for systemic toxicity in mice was 1600 ppm (equal to 153 mg/kg bw per day), and the overall LOAEL was 8000 ppm (equal to 787 mg/kg bw per day).

The Meeting concluded that there is equivocal evidence of induction of lymphomas in male mice in three out of seven studies (Sugimoto, 1997; Kumar, 2001; Wood et al., 2009a) and in female mice in one out of seven studies (Takahashi, 1999a) at high doses (5000–40 000 ppm, equal to 814–4348 mg/kg bw per day). The Meeting also noted that in the other three studies in which even higher doses (up to 50 000 ppm, equal to 7470 mg/kg bw per day) had been used, no effect was observed.

The Meeting concluded that there is some indication, by a trend test and not by pairwise comparison, of induction of kidney adenomas in male mice in four out of seven studies (Knezevich & Hogan, 1983; Sugimoto, 1997; Takahashi, 1999a; Kumar, 2001). The Meeting noted that the increases were marginal and occurred at the highest dose only and that other studies that used appreciably higher doses did not find any excess. However, the Meeting noted that kidney adenomas are uncommon in male mice.

Eleven combined chronic toxicity and carcinogenicity studies in rats were available (Lankas, 1981; Pavkov & Wyand, 1987; Strout & Ruecker, 1990; Atkinson et al., 1993b; Milburn, 1996; Suresh, 1996; Bhide, 1997; Enomoto, 1997; Takahasi, 1999a,b; Brammer, 2001; Wood et al., 2009b). One study was considered to be inadequate for carcinogenicity assessment due to its exposure duration (12 months). Toxicities variously reported in some of these studies included increased incidences of clinical signs, reduced body weights, degenerative lens changes (cataracts) in males, microscopic findings in the salivary gland, increased incidence of basophilia of parotid acinar cells, and microscopic findings in liver, prostate and kidneys. The overall NOAEL for systemic toxicity in rats was 100 mg/kg bw per day, and the overall LOAEL was 300 mg/kg bw per day.

The Meeting discussed the increased incidence of a variety of tumours observed in one or, in one case, two of the 10 studies in rats. The Meeting concluded that these findings were incidental, based on the following considerations:

- interstitial cell tumours of the testes: occurred in only one study (Lankas, 1981); and other studies that used appreciably higher doses did not find any excess;
- pancreatic islet cell adenoma: occurred in only one study in males only (Strout & Ruecker, 1990); other studies that used appreciably higher doses did not find any excess; there was no dose–response relationship; and the incidence in controls was unusually low (less than the lower bound of the historical control data); the Meeting also noted that there was a negative dose–response relationship in females;
- thyroid C-cell tumours: occurred in only one study (Strout & Ruecker, 1990); other studies that used appreciably higher doses did not find any excess; and these tumours are considered not to be relevant for humans;
- skin keratoma: occurred in two studies in males only; other studies that used appreciably higher doses did not find any excess; in one study (Strout & Ruecker, 1990), there was no dose–response relationship; and in the other study, only the test for trend was statistically significant, not the pairwise test at any dose (Enomoto, 1997); and
- lymphoma (in spleen and kidney): no evidence of induction in any of the studies.

The Meeting concluded that there is no reliable evidence for treatment-related tumours in rats at doses up to 32 000 ppm (equal to 1750 mg/kg bw per day).

The Meeting concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses.

Glyphosate and its formulation products have been extensively tested for genotoxic effects using a variety of tests in a wide range of organisms. While no mutational effects have been detected in bacterial test systems, DNA damage and chromosomal effects have commonly been seen in cell culture models and in organisms that are phylogenetically distant from humans. However, these effects have not been seen in vivo in orally treated mammalian models. The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg bw by the oral route, the route most relevant to human dietary exposure, was not associated with genotoxic effects in an overwhelming majority of studies conducted in mammals, a model considered to be appropriate for assessing genotoxic risks to humans.

The Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures.

Seven reproductive toxicity studies in rats were available. No evidence of reproductive toxicity was observed at doses up to 30 000 ppm (equal to 1983 mg/kg bw per day). In one study, an increased incidence of histopathological findings in the parotid (both sexes) and submaxillary salivary glands in females was observed in both generations at 10 000 ppm (equal to 668 mg/kg bw per day). The NOAEL was 3000 ppm (equal to 197 mg/kg bw per day) (Brooker et al., 1992). In a separate study, an increased incidence of loose stools and caecum distension was observed in both generations at 30 000 ppm (equal to 2150 mg/kg bw per day), and the NOAEL was 6000 ppm (equal to 417 mg/kg bw per day) (Takahashi, 1997). Slight reductions in pup weight or weight gain were observed in most studies, but were confined to very high, parentally toxic dose levels (Moxon, 2000; Takahashi, 1997). In addition, a significant delay in sexual maturation in male pups (F_1) was seen at 15 000 ppm (equal to 1063 mg/kg bw per day) (Dhinsa, 2007). The overall NOAEL for parental toxicity was 6000 ppm (equal to 417 mg/kg bw per day), and the overall LOAEL was 10 000 ppm (equal to 668 mg/kg bw per day). The overall NOAEL for offspring toxicity was 6000 ppm (equal to 417 mg/kg bw per day), and the overall LOAEL was 10 000 ppm (equal to 985 mg/kg bw per day).

No evidence of teratogenicity was observed in four developmental toxicity studies in rats at doses up to 3500 mg/kg bw per day. There was some variation in the extent of toxicity observed in the four studies. The lowest NOAEL for maternal toxicity was 300 mg/kg bw per day, based on loose stools and reduced body weights seen at 1000 mg/kg bw per day (Hatakenaka, 1995). The lowest NOAEL for embryo and fetal toxicity was 300 mg/kg bw per day, based on delayed ossification and an increased incidence of fetuses with skeletal anomalies observed at 1000 mg/kg bw per day.

Seven developmental toxicity studies in the rabbit were available. Maternal toxicity was primarily manifested as an increased incidence of soft stool and diarrhoea at doses of 175 mg/kg bw per day and above. The overall NOAEL for maternal toxicity was 100 mg/kg bw per day. In three studies, the occurrences of a variety of low-incidence fetal effects (e.g. cardiac malformation, absent kidney) were slightly increased at higher dose levels (Bhide & Patil, 1989; Brooker et al., 1991b; Suresh, 1993c). These increases are considered secondary to maternal toxicity. The overall NOAEL for embryo and fetal toxicity was 250 mg/kg bw per day (Bhide & Patil, 1989), based on effects at 450 mg/kg bw per day. The Meeting considered that these effects were secondary to local irritation from unabsorbed glyphosate in the colon administered by gavage dosing and concluded that they were not relevant for establishing health-based guidance values.

The Meeting concluded that glyphosate is not teratogenic.

Glyphosate was tested in a range of validated in vivo and in vitro assays for its potential to interact with the endocrine system. The studies that the Meeting considered adequate for the evaluation clearly demonstrate that there is no interaction with estrogen or androgen-receptor pathways or thyroid pathways.

There was no evidence of neurotoxicity in an acute neurotoxicity study in rats at doses up to 2000 mg/kg bw. The NOAEL for systemic toxicity was 1000 mg/kg bw, based on a single death and general signs of toxicity at 2000 mg/kg bw (Horner, 1996a). In a 90-day neurotoxicity study in rats,

no evidence of neurotoxicity or systemic toxicity was seen at doses up to 20 000 ppm (equal to 1546.5 mg/kg bw per day) (Horner, 1996b).

No evidence of immunotoxicity was seen in a 28-day dietary study in female mice at doses up to 5000 ppm (equal to 1448 mg/kg bw per day) (Haas, 2012).

Effects on the salivary glands were observed in several repeated-dose toxicity studies in rats. The pH of glyphosate in solution is low, and it has been shown that exposure to organic acids can cause such changes in salivary glands. Therefore, the changes are likely secondary to the effects caused by the pH of the test compound in solution.

In many of the long-term repeated-dose studies reviewed, glyphosate was reported to have an impact on the gastrointestinal tract at high doses. Although this is not uncommon with high-dose chemical substance administration, this was investigated further, as glyphosate is known to be poorly absorbed in mammalian models, and alterations in gut microbiota profiles, specifically reductions in the beneficial microbiota and increases in pathogenic bacteria, are known to have impacts on carcinogenesis. There is evidence from livestock species that pathogenic bacteria are more resistant to glyphosate, whereas beneficial microbiota are more sensitive, and thus more vulnerable.

This is an emerging area of scientific investigation. The extent to which glyphosate adversely affects the normal functioning of the microbiota in the human gastrointestinal tract or the gastrointestinal tract of mammalian models is unclear. However, it is unlikely, given the available information on MIC values, that this would occur from glyphosate residues in the diet.

Toxicological data on metabolites and/or degradates

AMPA is the only identified metabolite found in the urine and faeces of orally treated rats. AMPA was of low acute oral and dermal toxicity in rats (LD_{50} > 5000 [Leah, 1988; Cuthbert & Jackson, 1993a] and > 2000 mg/kg bw [Leuschner, 2002a], respectively) and was not sensitizing in guinea pigs, as determined by the Magnusson–Kligman maximization test. In a 90-day study of toxicity in rats, the NOAEL was 1000 mg/kg bw per day, the highest dose tested (Strutt et al., 1993). AMPA administered orally in mammalian test systems showed no evidence of genotoxicity (Leah, 1988; Cuthbert & Jackson, 1993a; Komura, 1996). Only negative results were seen in studies in vitro (Callander, 1988b; Jensen, 1993a; Akanuma, 1996). The Meeting concluded that AMPA is unlikely to be genotoxic in vivo by the oral route.

In a study of developmental toxicity in rats, no evidence for embryo or fetal toxicity was observed; the NOAEL for maternal and embryo/fetal toxicity was 1000 mg/kg bw per day, the highest dose tested.

Following single gavage administration of radiolabelled *N*-acetyl-glyphosate, a plant-specific metabolite, at 15 mg/kg bw in rats, about 66.1% of the administered dose was excreted in urine (61.3% within 12 hours post dosing), 26.4% in faeces (25.8% within 48 hours post dosing), 2.79% in cage wash and wipe, and 0.23% in residual carcass. Radioactivity was eliminated rapidly from blood and plasma, with half-life values of 20.1 and 15.6 hours, respectively. Unchanged [^{14}C]*N*-acetyl-glyphosate recovered in urine and faeces represented over 99% of the administered radioactivity. Glyphosate, a metabolite of *N*-acetyl-glyphosate, was detected in faeces and represented less than 1% of the total radioactivity (Cheng & Howard, 2004).

The acute oral toxicity LD_{50} of *N*-acetyl-glyphosate in rats is greater than 5000 mg/kg bw, expressed as the free acid (Vegarra, 2004). In a 90-day toxicity study in rats, the NOAEL was 18 000 ppm (equal to 1157 mg/kg bw per day) (MacKenzie, 2007).

N-Acetyl-glyphosate was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that *N*-acetyl-glyphosate is unlikely to be genotoxic.

N-Acetyl-AMPA, another plant-specific metabolite, was of low acute oral toxicity; the LD_{50} was greater than 5000 mg/kg bw in rats (Carpenter, 2007).

N-Acetyl-AMPA was tested for genotoxicity in vitro and in vivo in an adequate range of assays; it was not found to be genotoxic in mammalian or microbial test systems.

The Meeting concluded that *N*-acetyl-AMPA is unlikely to be genotoxic.

Human data

Routine medical surveillance of workers in production and formulation plants revealed no adverse health effects attributable to glyphosate. In operators applying glyphosate products, cases of eye, skin and/or respiratory tract irritation have been reported. Acute intoxication was reported in humans after accidental or intentional ingestion of concentrated glyphosate formulations, resulting in gastrointestinal, cardiovascular, pulmonary and renal effects and, occasionally, death. The acute toxicity of glyphosate formulations was likely caused by the surfactant in these products (JMPR, 2004).

Several epidemiological studies on cancer outcomes following occupational exposure to glyphosate were available. The evaluation of these studies focused on the occurrence of NHL, as outlined in Section 2.2 of the Meeting report. One meta-analysis and one prospective cohort study, the AHS, with a large sample size and detailed exposure assessment, were available. Cohort studies are considered a powerful design, as recall bias is avoided. All other studies were case–control studies, usually retrospective, which are more prone to recall and selection biases.

The AHS cohort study found no evidence of a positive association of NHL with glyphosate exposure or an exposure–response relationship (De Roos et al., 2005). Elevated risks were reported in various case–control studies. A significant elevated risk of NHL associated with ever- versus never-use of glyphosate (OR = 2.1; 95% CI = 1.1–4.0) was reported (De Roos et al., 2003). Ever-use of glyphosate was not associated with risk of NHL in the Cross-Canada Case–control Study of Pesticides and Health (McDuffie et al., 2001), but when analysing days of use per year, there was a significant elevated risk in the highest usage category (OR = 2.12; 95% CI = 1.20–3.73; for > 2 days/year glyphosate use). There was, however, no indication of an exposure–response relationship across exposure usage categories (McDuffie et al., 2001). In another case–control study, a significant increased risk of NHL associated with ever-use (OR = 2.02; 95% CI = 1.10–3.71) as well as the highest usage category (OR = 2.36; 95% CI = 1.04–5.37; for greater than 10 days/year glyphosate use) was observed, with some suggestion of an exposure–response gradient (Eriksson et al., 2008). Two smaller case–control studies with few exposed cases and limited statistical power reported a nonsignificant elevated risk (Hardell et al., 2002) and no association (Orsi et al., 2009), respectively, for risk of NHL and ever-use of glyphosate. The meta-analysis, including the AHS, found a significant 50% excess risk ratio for ever- versus never-use of glyphosate (Schinasi & Leon, 2014).

Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta-analysis. However, it is notable that the AHS (De Roos et al., 2005), which is the only cohort study and is large and of high quality, found no evidence of association at any exposure level.

In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans via exposure from the diet.

The Meeting concluded that the existing database on glyphosate was adequate to characterize the potential hazards to the general population, including fetuses, infants and children.

Toxicological evaluation

The Meeting reaffirmed the group ADI for the sum of glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA of 0–1 mg/kg bw on the basis of the NOAEL of 100 mg/kg bw per day for effects on the salivary gland in a long-term study of toxicity and carcinogenicity in rats and

application of a safety factor of 100. The Meeting noted that these effects may be secondary to local irritation due to the low pH of glyphosate in solution, but was unable to establish this unequivocally.

The Meeting concluded that it was not necessary to establish an ARfD for glyphosate, AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA in view of their low acute toxicity, the absence of relevant developmental toxicity in rats and rabbits that could have occurred as a consequence of acute exposure, and the absence of any other toxicological effect that would be elicited by a single dose.

Levels relevant to risk assessment of glyphosate

Species	Study	Effect	NOAEL	LOAEL
Mouse	Eighteen- to 24-month studies of toxicity and carcinogenicity[a,b]	Toxicity	1 600 ppm, equal to 153 mg/kg bw per day[c]	8 000 ppm, equal to 787 mg/kg bw per day
		Carcinogenicity	The Meeting could not exclude the possibility that glyphosate is carcinogenic in mice at very high doses.	
Rat	Acute neurotoxicity study[a]	Neurotoxicity	2 000 mg/kg bw[c]	–
	Two-year studies of toxicity and carcinogenicity[b]	Toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Carcinogenicity	32 000 ppm, equal to 1 750 mg/kg bw per day[c]	–
	Two-generation studies of reproductive toxicity[a,b]	Reproductive toxicity	30 000 ppm, equal to 1 983 mg/kg bw per day[c]	–
		Parental toxicity	6 000 ppm, equal to 417 mg/kg bw per day	10 000 ppm, equal to 668 mg/kg bw per day
		Offspring toxicity	6 000 ppm, equal to 417 mg/kg bw per day	10 000 ppm, equal to 985 mg/kg bw per day
	Developmental toxicity studies[b,d]	Maternal toxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
		Embryo and fetal toxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
Rabbit	Developmental toxicity studies[b,d]	Maternal toxicity[e]	100 mg/kg bw per day	175 mg/kg bw per day
		Embryo and fetal toxicity[e]	250 mg/kg bw per day	450 mg/kg bw per day
Dog	Thirteen-week and 1-year studies of toxicity[b,f]	Toxicity	15 000 ppm, equal to 448 mg/kg bw per day	30 000 ppm, equal to 926 mg/kg bw per day
AMPA				
Rat	Thirteen-week study of toxicity[d]	Toxicity	1 000 mg/kg bw per day[c]	–
	Developmental toxicity study[d]	Maternal toxicity	1 000 mg/kg bw per day[c]	–
		Embryo and fetal toxicity	1 000 mg/kg bw per day[c]	–

[a] Dietary administration.
[b] Two or more studies combined.
[c] Highest dose tested.
[d] Gavage administration.
[e] Secondary to local irritation of the colon.
[f] Capsule administration.

504

Estimate of acceptable daily intake (ADI)

0–1 mg/kg bw (for sum of glyphosate, *N*-acetyl-glyphosate, AMPA and *N*-acetyl-AMPA)

Estimate of acute reference dose (ARfD)

Unnecessary

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to glyphosate

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapidly, but only to a limited extent (about 20–30%)
Dermal absorption	About 1–3%
Distribution	Widely distributed (low levels occurring in all tissues)
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapid and nearly complete in 48 h (about 20–30% in urine and about 60–70% in faeces)
Metabolism in animals	Very limited (< 0.7%), by hydrolysis leading to AMPA
Toxicologically significant compounds in animals and plants	Parent compound, AMPA, *N*-acetyl-glyphosate, *N*-acetyl-AMPA
Acute toxicity	
Rat, LD_{50}, oral	5 600 mg/kg bw
Rat, LD_{50}, dermal	> 2 000 mg/kg bw
Rat, LC_{50}, inhalation	> 5.48 mg/L
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Moderately to severely irritating
Guinea-pig, dermal sensitization	Not sensitizing (Magnusson and Kligman test, Buehler test)
Mouse, dermal sensitization	Not sensitizing (local lymph node assay)
Short-term studies of toxicity	
Target/critical effect	Clinical signs (loose stools, diarrhoea), liver, salivary glands and reduced body weights
Lowest relevant oral NOAEL	300 mg/kg bw per day (90 days; rat)
Lowest relevant dermal NOAEL	> 5 000 mg/kg bw per day (21 days; rabbit)
Lowest relevant inhalation NOAEC	No data
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body weights, loose stools, liver (toxicity), salivary glands (organ weight, histology), eye (cataracts, lens fibre degeneration)
Lowest relevant NOAEL	100 mg/kg bw per day (2 years; rat)
Carcinogenicity	Not carcinogenic in rats; could not exclude possibility of carcinogenicity in mice at very high doses[a]
Genotoxicity	

505

Absorption, distribution, excretion and metabolism in mammals	
	No genotoxic potential via oral route in mammals[a]
Reproductive toxicity	
Target/critical effect	Reduced body weights and delayed development (absence of maternal toxicity)
Lowest relevant parental NOAEL	417 mg/kg bw per day (rat)
Lowest relevant offspring NOAEL	417 mg/kg bw per day (rat)
Lowest relevant reproductive NOAEL	1 983 mg/kg bw per day (rat)
Developmental toxicity	
Target/critical effect	Slight increase in malformations at maternally toxic doses
Lowest relevant maternal NOAEL	100 mg/kg bw per day (rabbit)[b]
Lowest relevant embryo/fetal NOAEL	250 mg/kg bw per day (rabbit)[b]
Neurotoxicity	
Acute neurotoxicity NOAEL	2 000 mg/kg bw, highest dose tested
Subchronic neurotoxicity NOAEL	1 547 mg/kg bw per day, highest dose tested
Developmental neurotoxicity NOAEL	No data
Other toxicological studies	
Immunotoxicity	No immunotoxicity; NOAEL 1 448 mg/kg bw per day, highest dose tested (28 days; mouse)
Studies on toxicologically relevant metabolites	Toxicological studies on AMPA, *N*-acetyl-glyphosate and *N*-acetyl-AMPA reveal the metabolites to be less toxic than the parent compound
Human data	
	Medical surveillance of workers in plants producing and formulating glyphosate did not reveal any adverse health effects. In operators applying glyphosate products, cases of eye, skin and/or respiratory irritation have been reported. Cases of acute intoxication have been observed after accidental or intentional ingestion of glyphosate formulation.

[a] Unlikely to pose a carcinogenic risk to humans via exposure from the diet.

[b] Secondary to local irritation of the colon.

Summary

	Value	Study	Safety factor
ADI	0–1 mg/kg bw	Two-year studies of toxicity (rat)	100
ARfD	Unnecessary	–	–

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515

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529

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Appendix 1(a). Results of in vitro genotoxicity with glyphosate in nonmammalian species

End-point	Test object	Concentration	Purity %	GLP (Yes/No)	Results	Reference
Chromosome damage	*Allium* root cells	w/o S9; 720–2880 µg/L	Glyphosate isopropylamine (96%)	No	Negative	Rank et al. (1993)
Chromosome alterations	*Allium* root cells	w/o S9; 720–2 880 µg/L calculated as glyphosate isopropylamine	Roundup	No	Positive	Rank et al. (1993)
Chromosome alterations	*Allium cepa* onion root tips	0.036– 0.146%	Springbok, glyphosate isopropylamine formulation (48%)	No	Positive	Asita & Makhalemele (2008)
Chromosome alterations	*Trigonella foenum-graecumn* root tips	0.1–0.5%	Glyphosate	No	Positive	Siddiqui et al. (2012)
Chromosome alterations	*Allium cepa* onion root tips	3%	Glyphosate	No	Positive	Frescura et al. (2013)
Micronucleus	*Allium cepa* onion root tips	35. 70, 105, 140, 350, 700. 1050 and 1400 µg/g	Glyphosate formulation (21%)	No	Equivocal	De Marco et al. (1992)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L	Glyphosate	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L active ingredient	Roundup	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	Frog (*Eleutherodactyl us johnstonei*) blood cells	4.6–37 mg a.e./cm^2	Roundup SL–Cosmoflux 411F (360 g/L glyphosate)	No	Positive	Meza-Joya, Ramirez-Pinilla & Fuentes-Lorenzo (2013)
DNA strand breaks (Comet assay)	Tilapia (*Oreochromis niloticus*) erythrocytes	w/o S9; 0.000 7– 0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2014)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Inconclusi ve	Alvarez-Moya et al. (2014)

S9: 9000 × *g* supernatant fraction

Appendix 1(b). Results of in vivo genotoxicity with glyphosate, Roundup and other formulations in nonmammalian species

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Glyphosate						
Mutation	*Drosophila larvae*	0.1 ppm	Pondmaster	N/S	Positive	Kale et al. (1995)
Mutation	*Drosophila larvae*	1 ppm	Roundup	N/S	Positive	Kale et al. (1995)
Mutation	*Drosophila* standard cross	0.1–10 mmol/L	Glyphosate (96%)	No	Weak positive	Kaya et al. (2000)
Mutation	*Drosophila* high bioactivation cross	0.1–10 mmol/L	Glyphosate (96%)	No	Negative	Kaya et al. (2000)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L	Glyphosate	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	17.9 35.7 µg/L	Glyphosate	No	Positive	Guilherme et al. (2012a)
DNA strand breaks (Comet assay)	Nile tilapia *Oreochromis niloticus* erythrocytes	w/o S9; 0.000 7–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Positive	Alvarez-Moya et al. (2014)
DNA strand breaks (Comet assay)	Spiderwort plant *Tradescantia* stamen hair nuclei	w/o S9; 0.0007–0.7 mmol/L	Glyphosate isopropylamine (96%)	No	Weak positive /inclusive	Alvarez-Moya et al. (2014)
DNA damage	Zebrafish (*Danio rerio*) sperm	5 & 10 mg/L	Glyphosate	No	Positive	Lopes et al. (2014)
DNA strand breaks (Comet assay)	Sabalo fish (*Prochilodus lineatus*) erythrocytes and gill cells	0.48 & 2.4 mg/L	Glyphosate	No	Positive	Moreno, Sofia & Martinez (2014)
Mutation (sex-linked recessive lethal)	*Drosophila* standard cross	1 ppm	Roundup	No	Positive	Kale et al. (1995)
Mutation (sex-linked recessive lethal)	*Drosophila* standard cross	0.1 ppm	Pondmaster	No	Positive	Kale et al. (1995)
Chromosomal aberrations	Plant meristems of *Crepis capillaris*	0.05–1%	Roundup (> 90% purity)	No	Negative	Dimitrov et al. (2006)
Chromosome abnormalities	Mitotic plant meristems of *Hordeum vulgare*	0.1–2%	Roundup	No	Positive	Truta et al. (2011)
Micronucleus	Nile tilapia fish *Oreochromis niloticus* erythrocytes	42–170 mg/kg bw	Glyphosate (Roundup 69)	N/S	Negative	Nascimento & Grisolia (2000)

536

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Micronucleus	*Tilapia rendalli* peripheral erythrocytes	42–170 mg/kg bw	Roundup (480 g/L)	No	Positive	Grisolia (2002)
Micronucleus	Plant meristems of *Crepis capillaris*	0.05–1%	Roundup (> 90% purity)	No	Negative	Dimitrov et al. (2006)
Micronucleus	The freshwater goldfish (*Carassius auratus*) erythrocytes	5, 10 and 15 ppm	Roundup (480 g/L)	No	Positive	Cavas & Konen (2007)
Micronucleus	Neotropical fish (*Prochilodus lineatus*) erythrocytes and gill cells	10 mg/L	Roundup (41%)	No	Negative	Cavalcante, Martinez & Sofia (2008)
Micronucleus	*Caiman latirostris* erythrocytes	50–1 750 µg/egg	Roundup (66.2%)	No	Positive	Poletta et al. (2009)
Micronucleus	European eel (*Anguilla anguilla*) blood cells	58 & 116 µg/L	Roundup (30.8%)	No	Negative	Guilherme et al. (2010)
Micronucleus	*Caiman latirostris* erythrocytes	3%	Roundup (66.2%)	No	Positive	Poletta et al. (2011)
Micronucleus and Nuclear abnormalities	Brazilian freshwater fish *Astyanax* sp.	0.006 mL/L	Roundup	No	Positive	Rossi et al. (2011)
Micronucleus	The fish *Corydoras paleatus* erythrocytes	6.67 µg/L	Roundup (48%)	No	Negative	De Castilhos, Ghisi & Cestari (2013)
Micronucleus	Guppy (*Poecilia reticulata*) gill erythrocytes	0, 1.41, 2.83, 4.24 and 5.65 µL/L	Roundup Transorb (64.8%)	No	Positive	De Souza Filho et al. (2013)
Micronucleus	*Caiman latirostris* erythrocytes	2.5–21 mg/L	Roundup	No	Positive	López Gonzáles et al. (2013)
Micronucleus	Ten spotted live-bearer fish *Cnesterodon decemmaculatus* erythrocytes	22.9–68.8 mg/L	Glyphosate formulation Credit (48%)	No	Positive	Vera-Candioti, Soloneski & Larramendy (2013)
Micronucleus	Ten spotted fish *Cnesterodon decemmaculatus* erythrocytes	3.9–11.8 mg/L	Glyphosate formulation Panzer (48%)	No	Weak positive	Vera-Candioti, Soloneski & Larramendy (2013)
Micronucleus	Indian skittering frog (*Euflictis cyanophlyctis*) tadpole erythrocytes	1–8 mg a.e./L	Roundup (41%)	No	Positive	Yadav et al. (2013)
Micronucleus	Earthworm (*Pheretima peguana*) coelomocytes	47–432 µg cm^{-2}	Glyphosate formulation (36%)	No	Positive	Muangphra, Kwankua & Gooneratne (2012)
Micronucleus	*Channa punctatus* blood cells	8.1–24.4 mg/L	Roundup (41%)	No	Positive	Nwani et al. (2014)

537

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Micronuclei and meiotic anomalies	Black lentil beans *Vigna mungo*	Not specified	Glyphosate	No	Positive	Singh & Srivastava (2014)
DNA strand breaks (Comet assay)	Bullfrog (*Rana catesbeiana*) tadpoles	1.69–27 mg/L	Roundup (356 g/L)	No	Positive	Clements, Ralph & Petras (1997)
DNA strand breaks (Comet assay)	Freshwater mussels (*Utterbackia imbecillis*)	2.5 and 5 mg/L	Roundup (18%)	No	Negative	Conners & Black (2004)
DNA strand breaks (Comet assay)	Freshwater goldfish (*Carassius auratus*) erythrocytes	5, 10 and 15 ppm	Roundup (480 g/L)	No	Positive	Cavas & Konen (2007)
DNA strand breaks (Comet assay)	Neotropical fish (*Prochilodus lineatus*) erythrocytes and gill cells	10 mg/L	Roundup (41%)	No	Weak positive	Cavalcante, Martinez & Sofia (2008)
DNA strand breaks (Comet assay)	*Caiman latirostris* erythrocytes	50–1 750 µg/egg	Roundup (66.2%)	No	Positive	Poletta et al. (2009)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2010)
DNA strand breaks (Comet assay)	*Caiman latirostris* erythrocytes	3%	Roundup (66.2%)	No	Positive	Poletta et al. (2011)
DNA strand breaks (Comet assay)	Snail (*Biomphalaria alexandrina*) haemocytes	10 mg/L	Roundup (48%)	No	Positive	Mohamed (2011)
DNA strand breaks (Comet assay)	Oyster spermatozoa	0.5; 1.0; 1.5; 2.5; 5.0 µg/L active ingredient	Roundup	No	Negative	Akcha, Spagnol & Rouxel (2012)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) gill and liver cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2012b)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2012a)
DNA strand breaks (Comet assay)	Guppy (*Poecilia reticulata*) gill erythrocytes	0, 1.41, 2.83, 4.24 and 5.65 µL/L	Roundup Transorb (64.8%)	No	Positive	De Souza Filho et al. (2013)
DNA strand breaks (Comet assay)	Frog (*Eleutherodactylus johnstonei*) blood cells	0.5–1.7 mg a.e./cm^2	Roundup SL–Cosmoflux 411F (360 g/L glyphosate)	No	Positive	Meza-Joya, Ramirez-Pinilla & Fuentes-Lorenzo (2013)
DNA strand breaks (Comet assay)	Fish *Corydoras paleatus* erythrocytes	6.67 µg/L	Roundup (48%)	No	Positive	De Castilhos, Ghisi & Cestari (2013)

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
DNA strand breaks (Comet assay)	Freshwater clam (*Corbicula fluminea*) haemocytes	2 and 10 ppm	Roundup	No	Negative	Dos Santos & Martinez (2014)
DNA strand breaks (Comet assay)	Common carp (*Cyprinus carpio*) erythrocytes	2 mg/L	Roundup (480 g/L)	No	Positive	Gholami-Seyedkolaei et al. (2013)
DNA strand breaks (Comet assay)	*Channa punctatus* blood and gill cells	3.25–6.51 mg/L	Roundup (41%)	No	Positive	Nwani et al. 2013
DNA strand breaks (Comet assay)	Earthworm (*Eisenia andrei*) coelomocytes	15 and 30 $\mu g/cm^{-1}$	Roundup FG (71%)	No	Positive	Piola et al. (2013)
DNA strand breaks (Comet assay)	Earthworm (*Eisenia andrei*) coelomocytes	15–240 $\mu g/cm^{-1}$	Glyphosate formulation (85.4%)	No	Negative	Piola et al. (2013)
DNA strand breaks (Comet assay)	Ten spotted live-bearer fish *Cnesterodon decemmaculatus* erythrocytes	3.9 mg/L	Glyphosate formulation Panzer (48%)	No	Positive	Vera-Candioti Soloneski & Larramendy (2013b)
DNA strand breaks (Comet assay)	Ten spotted live-bearer fish *Cnesterodon decemmaculatus* erythrocytes	22.9 mg/L	Glyphosate formulation Credit (48%)	No	Positive	Vera-Candioti, Soloneski & Larramendy (2013b)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	116 µg/L	Roundup (30.8%)	No	Positive	Guilherme et al. (2014a)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) liver cells	58 and 116 µg/L	Roundup (30.8%)	No	Positive	Marques et al. (2014)
DNA strand breaks (Comet assay)	Sabalo fish (*Prochilodus lineatus*) erythrocytes and gill cells	1 and 5 mg/L	Roundup Transorb (480 g/L)	No	Positive	Moreno, Sofia & Martinez (2014)
DNA strand breaks (Comet assay)	Earthworm (*Pheretima peguana*) coelomocytes	47–432 µg cm^{-2}	Glyphosate formulation (36%)	No	Negative	Muangphra Kwankua & Gooneratne (2012)
DNA strand breaks (Comet assay)	Tambaqui (Colossoma macropomum) fish	10–15 mg/L	Roundup (360 g/L)	No	Positive	Braz-Mota et al. (2015)
DNA breakage (Comet assay)	Neotropical fish *Prochilodus lineatus* blood cells	0.15–1.5 mg/L	Polyoxyethylene amine	N/S	Positive	Navarro & Martinez (2014)
AMPA						
Micronucleus	European eel (*Anguilla anguilla*) blood cells	11.8, 23.6 µg/L	N/A	No	Negative	Guilherme et al. (2014b)
DNA strand breaks (Comet assay)	European eel (*Anguilla anguilla*) blood cells	11.8, 23.6 µg/L	N/A	No	Positive	Guilherme et al. (2014b)

End-point	Test object	Concentration	Purity (%)	GLP (Yes/No)	Results	Reference
Nuclear abnormalities	European eel (*Anguilla anguilla*) blood cells	11.8, 23.6 µg/L	N/A	N/S	Positive	Guilherme et al. (2014b)

AMPA: aminomethylphosphonic acid; bw: body weight; GLP: good laboratory practice; N/A: not applicable; N/S: not stated; ppm: parts per million; S9: 9000 × g supernatant fraction

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541

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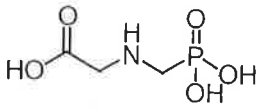
403 glyphosate 542

Herbicide

Target Site EPSP synthase inhibition (5-enolpyruvylshikimate-3-phosphate)
HRAC G **WSSA** 9 *glycine derivative*



NOMENCLATURE:
glyphosate
Common name glyphosate (BSI, E-ISO, (*m*) F-ISO, ANSI, WSSA, JMAF)
IUPAC name *N*-(phosphonomethyl)glycine
Chemical Abstracts name *N*-(phosphonomethyl)glycine
CAS RN *[1071-83-6]* **EC no.** 213-997-4 **EPA PC** 417300 **Development codes** CP [illegible] (Monsanto); MON 0573 (Monsanto)

glyphosate-diammonium
IUPAC name diammonium *N*-(phosphonatomethyl)glycine
Chemical Abstracts name *N*-(phosphonomethyl)glycine ammonium salt (1:2)
CAS RN *[69254-40-6]* **EPA PC** 103607

glyphosate-dimethylammonium
IUPAC name dimethylammonium *N*-(phosphonomethyl)glycinate; *N*-(phosphonomethyl)glycine – dimethylamine (1:1)
Chemical Abstracts name *N*-(phosphonomethyl)glycine compound with *N*-methylmethanamine (1:1)
CAS RN *[34494-04-7]* **EPA PC** 103608

glyphosate-isopropylammonium
IUPAC name isopropylammonium *N*-(phosphonomethyl)glycinate; *N*-(phosphonomethyl)glycine – isopropylamine (1:1)
Chemical Abstracts name *N*-(phosphonomethyl)glycine compound with 2-propanamine
CAS RN *[38641-94-0]* **EC no.** 254-056-8 **EPA PC** 103601
Development codes MON 77209 (Monsanto); MON 0139 (Monsanto)

glyphosate-monoammonium
IUPAC name ammonium *N*-[(hydroxyphosphinato)methyl]glycine
Chemical Abstracts name *N*-(phosphonomethyl)glycine ammonium salt (1:1)
CAS RN *[40465-66-5]*
Other names glyphosate-ammonium **Development codes** MON 8750 (Monsanto)

glyphosate-potassium
IUPAC name potassium *N*-[(hydroxyphosphinato)methyl]glycine
Chemical Abstracts name *N*-(phosphonomethyl)glycine potassium salt (1:1)
CAS RN *[39600-42-5]*

glyphosate-sesquisodium
Chemical Abstracts name *N*-(phosphonomethyl)glycine sodium salt (2:3)
CAS RN *[70393-85-0]* **EPA PC** 103603 **Development codes** MON 8722 (glyphosate-sodium) (Monsanto); MON 8000 (Monsanto)

PHYSICAL CHEMISTRY:

glyphosate

Composition Tech. is ≥95%. Zwitterion structure (P. Knuuttilä & H. Knuuttila, *Acta Chem. Scand.*, Ser. B, **33**, 623). **M.f.** $C_3H_8NO_5P$ **Mol. wt.** 169.1 **Physical form** Odourless, white crystals. **M.p. (°C)** 189.5 **V.p. (mPa)** 0.0131 (25 °C) **Henry (Pa m^3 mol^{-1}, calc.)** $<2.1 \times 10^{-7}$ **log K_{ow}** <-3.2 (pH 5-9). **pKa (20–25 °C)** 2.34; 5.73; 10.2 **Water solubility (mg/l, 20–25 °C)** 1.05×10^4 (pH 1.9) **Organic solubility (g/l, 20–25 °C)** Soluble in acetone (0.078), dichloromethane (0.233), ethyl acetate (0.012), methanol (0.231), isopropanol (0.02), toluene (0.036) **F.p.** Not flammable **S.g./Bulk density (20–25 °C)** 1.704 **Stability** Glyphosate and all its salts are non-volatile, do not photochemically degrade in buffered water and are stable in air. Glyphosate is stable to hydrolysis at pH 3, 6 and 9 (5–35 °C).

glyphosate-diammonium

M.f. $C_3H_{14}N_3O_5P$ **Mol. wt.** 203.1

glyphosate-dimethylammonium

M.f. $C_5H_{15}N_2O_5P$ **Mol. wt.** 214.2

glyphosate-isopropylammonium

M.f. $C_6H_{17}N_2O_5P$ **Mol. wt.** 228.2 **Physical form** Odourless, white powder. **M.p. (°C)** 143–164; 189–223 (dimorphic) **V.p. (mPa)** 0.0021 (25 °C) **Henry (Pa m^3 mol^{-1}, calc.)** 4.6×10^{-10} **log K_{ow}** −5.4 **pKa (20–25 °C)** 2.18; 5.77 **Water solubility (mg/l, 20–25 °C)** 1.05×10^6 (pH 4.3) **Organic solubility (g/l, 20–25 °C)** Soluble in ethyl acetate (0.00004), heptanes (0.00004), methanol (15.7–28.4) **B.p.** Decomp. **S.g./Bulk density (20–25 °C)** 1.482 **Stability** Stable 5 days at pH 4, 5 and 9 (50 °C).

glyphosate-monoammonium

Composition Tech. is 95.2%. **M.f.** $C_3H_{11}N_2O_5P$ **Mol. wt.** 186.1 **Physical form** Odourless, white powder. **M.p. (°C)** >190 (decomp.) **V.p. (mPa)** 0.009 (25 °C) **Henry (Pa m^3 mol^{-1}, calc.)** 1.16×10^{-8} **log K_{ow}** <-3.7 **pKa (20–25 °C)** 5.5 **Water solubility (mg/l, 20–25 °C)** 1.44×10^5 (pH 3.2) **Organic solubility (g/l, 20–25 °C)** Soluble in acetone (0.0023), methanol (0.159) **F.p.** Not flammable **S.g./Bulk density (20–25 °C)** 1.433 **Stability** Stable over 5 days at 50 °C (pH 4, 7 and 9).

glyphosate-potassium

Composition In products described as containing glyphosate-potassium, the CAS Registry Number for the salt with unspecified potassium content *[70901-12-1]* is sometimes quoted. **M.f.** $C_3H_7KNO_5P$ **Mol. wt.** 207.2 **M.p. (°C)** 219.8 **Henry (Pa m^3 mol^{-1}, calc.)** 3.38×10^{-7} **log K_{ow}** <-4.0 **pKa (20–25 °C)** 5.7 **Water solubility (mg/l, 20–25 °C)** 9.187×10^5 (pH 7) **Organic solubility (g/l, 20–25 °C)** Soluble in methanol (0.217)

glyphosate-sesquisodium

M.f. $C_6H_{14}N_2Na_3O_{10}P_2$ **Mol. wt.** 405.2 **Physical form** Odourless, white powder. **M.p. (°C)** >260 (decomp.) **V.p. (mPa)** 0.00756 (25 °C) **Henry (Pa m^3 mol^{-1}, calc.)** 4.27×10^{-10} **log K_{ow}** −4.58 **Water solubility (mg/l, 20–25 °C)** 4.14×10^5 (pH 4.2) **Stability** Stable over 5 days (pH 4, 7 and 9, 50 °C).

COMMERCIALISATION: **History** Herbicidal activity reported by D. D. Baird *et al.* (*Proc. North Cent. Weed Control Conf.*, 1971, **26**, 64). Salts were introduced by Monsanto Co. in 1974. **Patents** US 3799758. **Manufacturers** Aako; ACA; Adama; Agrochem; AgroDragon; Aimco; Arcom; Anhui Huaxing; Anpon; Atanor; Bailing; Baocheng; Binnong; CAC; Changxing First;

Chongqing Shuangfeng; Comlets; Dow AgroSciences; Drexel; Excel Crop Care; Fengle; Fengshan; Fertiagro; FMC; Guangxin Agrochemical; Hailir; Hebei Golhil; Heranba; High Kite; Hindustan; Hui Kwang; Jiangsu Good Harvest; Jiangsu Inter-China Group Corporation; Jiangsu Kuaida; Jiangsu Yangnong; Jinfanda; Jingbo; Jingma; Krishi Rasayan; KSA; Labor; Lianyungang Liben; Meghmani; Modern Insecticides; Monsanto; Nantong Jiangshan; Nortox; Nufarm China; Nufarm GmbH; Nufarm Ltd; Nufarm SAS; Nufarm UK; Pyosa; Rainbow; Ranjit; Sannong; Sega; Shandong Qiaochang; Shanghai Kaipu; Sharda; Sinon; Sundat; Sunjoy; UPL; Wintafone; Wynca; Xianlong; Zhejiang Biok; Zhejiang Linghua

APPLICATIONS:

glyphosate

Spectrum and Route of Action Non-selective systemic herbicide, absorbed by the foliage, with rapid translocation throughout the plant. Inactivated on contact with soil. **Uses** Post-emergence control of annual and perennial grass and broad-leaved weeds in genetically engineered, glyphosate-tolerant soybeans, maize, canola, alfalfa, sugar beets, and cotton; post-emergence control of annual and perennial grass and broad-leaved weeds, pre-harvest, in cereals, peas, beans, oilseed rape, flax and mustard, at 1500–2000 g/ha; control of annual and perennial grass and broad-leaved weeds in stubble and post-planting/pre-emergence in many crops; control of annual and perennial grass and broad-leaved weeds in vines and olives, at up to 4300 g/ha as a directed spray application; control of annual and perennial grass and broad-leaved weeds in orchards, pastures, forestry and industrial and around households, at up to 4300 g/ha; pre-harvest desiccation in sugar cane and cereals; control of aquatic weeds, at 2000 g/ha.
Formulation types SG; SL **Compatibility** Mixing with other herbicides may reduce the activity of glyphosate. **Site of Action** Inhibits 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme of the aromatic acid biosynthetic pathway. This prevents synthesis of essential aromatic amino acids needed for protein biosynthesis. **Selected products** Coneo (Atul); Gladiator (Arysta India); Glyfall (Hermoo); Karda (Lainco); Maxweed (Crop Health); Nasa (Agria); PI Glypho (P I Industries); Pilarsato (Pilarquim); Prince (Aako); Rinder (Inquiport); Rophosate (Rotam); Seccherba (Agrimix); Sharp (Baocheng) **Selected mixtures** FlexstarGT (Syngenta); Folar (Syngenta); Halex GT (Syngenta); Ovni (Aako)

glyphosate-sesquisodium

Uses Sugar cane ripener.

ANALYSIS: **Product** analysis by hplc with uv detection (*AOAC Methods*, 18th Ed., 983.10; *CIPAC Handbook*, 1985, **1C**, 2132; *ibid.*, 1998, **H**, 182), or by ion-exchange lc with uv detection (*AOAC Methods*, 18th Ed., 996.12). **Residues** determined by gc with MSD (also applicable to aminomethylphosphonic acid, *AOAC Methods*, 18th Ed., 2000.05), by hplc with *o*-phthalaldehyde post-column reaction specific for primary amines (*J. Agric. Food Chem.*, 1986, **34**(6), 955–960), or by gc/FPD (*Resid. Anal. Methods*). See also *Pestic. Anal. Man.*, **II**, 180.364. In **water** by hplc determination by *o*-phthalaldehyde post-column reaction system (*AOAC Methods*, 18th Ed., 991.08), by hplc/FLD (*Environ. Chem. Methods*), or by gc/FPD (*ibid.*). In **soil** by gc/FPD or gc/ms (*ibid.*).

REGULATORY: **Toxicological & Environmental Reviews** *EHC* 159 (1994); *JMPR Mtg.* (1994), (1997), (2004), (2005), (2011), (2013), (2016); *JMPR Evaln. I* (1994), (1997), (2005), (2011), (2013); *JMPR Evaln. II* (1986), (1997), (2004), (2011), (2016); *ICSC* 0160 (2005); *IARC* 112 (2015); *EU Rev. Rep.* 6511/VI/99 (2002); *EFSA Jou.* 2015, **13**(11), 4302; *EPA Fact Sheet*, Sep. 1993; *EPA RED*, Sep. 1993. **EU status** Approved **Legislation** (EU) 2017/2324, (EU) 540/2011
EPA Status Registered – Registration Under Review **IARC class** 2A **Water GV (µg/l)** Not established **Toxicity class: WHO (a.i.)** III

MAMMALIAN TOXICOLOGY:

glyphosate

Acute oral (LD_{50}, mg/kg) rats >5000; mice >10000 **Acute dermal (LD_{50}, mg/kg)** rabbits >5000 **Acute Inhalation (LD_{50}, mg/l)** rats >4.98 (4 h). **Skin irritation** Not an irritant (rabbits) **Skin sensitisation** Not a sensitiser (guinea pigs) **Eye** Irritant (rabbits) **NOEL** In 2 y feeding trials, no ill-effects were observed in rats receiving 410 mg/kg diet daily (ave.) and, in 1 y feeding trials, no ill-effects were observed in dogs receiving 500 mg/kg daily (highest dose treated). Lowest relevant NOAEL (2 y) for rats 31 mg/kg b.w. daily (EU). **ADI/RfD** (JMPR) ADI 1 mg/kg b.w. [2016]; (EFSA) ADI 0.5, aRfD 0.5, AOEL 0.1 mg/kg b.w. [2015]; (EPA) cRfD 2 mg/kg b.w. [1993]. **Other** Not mutagenic, not carcinogenic, not teratogenic, not neurotoxic. No adverse effects on reproduction.

glyphosate-isopropylammonium

Acute oral (LD_{50}, mg/kg) rats >5000 **Acute dermal (LD_{50}, mg/kg)** rabbits >5000 **Acute Inhalation (LD_{50}, mg/l)** rats >1.3 (4 h) **Skin irritation** Not an irritant (rabbits) **Eye** Slight irritant (rabbits) **NOEL** In a 6 mo capsule trial, no ill-effects were observed in dogs receiving 300 mg/kg daily (highest dose treated).

glyphosate-monoammonium

Acute oral (LD_{50}, mg/kg) rats 4610 **Acute dermal (LD_{50}, mg/kg)** rabbits >5000 **Acute Inhalation (LD_{50}, mg/l)** rats >1.9 **Skin irritation** Not an irritant (rabbits) **Eye** Slight irritant (rabbits)

glyphosate-potassium

Acute oral (LD_{50}, mg/kg) rats >5000 **Acute dermal (LD_{50}, mg/kg)** rats >5000 **Acute Inhalation (LD_{50}, mg/l)** rats >5.27 (4 h) **Skin irritation** Not an irritant (rabbits) **Eye** Irritant (rabbits)

glyphosate-sesquisodium

Acute dermal (LD_{50}, mg/kg) rats >5000 **Skin irritation** Not an irritant (rabbits) **Eye** Slight irritant (rabbits)

ECOTOXICOLOGY:

glyphosate

Birds Acute oral LD_{50} for bobwhite quail >3851 mg/kg. Dietary LC_{50} (5 d) for quail and ducks >4640 mg/kg diet. **Fish** LC_{50} (96 h) for rainbow trout 38, bluegill sunfish 47, zebrafish 123, carp >1000 mg/l. **Daphnia** LC_{50} (48 h) 40 mg/l. **Algae** E_bC_{50} (72 h) for *Selenastrum capricornutum* 485 mg/l, E_rC_{50} (72 h) for *S. capricornutum* 460 mg/l; EC_{50} (96 h) for *Skeletonema costatum* 1.3 mg/l; EC_{50} (7 d) for *Navicula pelliculosa* 42, *Anabaena flos-aquae* 15 mg/l. **Other aquatic spp.** LC_{50} (96 h) for mysid shrimps >1000, grass shrimps 281, fiddler crabs 934 mg/l; EC_{50} (96 h) for sea urchins >1000 mg/l; (14 d) for *Lemna gibba* 12 mg/l; (48 h) for *Litoria moorei* tadpoles 111 mg/l. **Bees (LD_{50}, µg/bee)** 100 (oral) (48 h); >100 (contact) (48 h) **Worms** LC_{50} (14 d) for *Eisenia foetida* 5600 mg/kg soil. **Other beneficial spp.** Harmless to slightly harmful to green lacewing, parasite species, mites/spiders and insects; moderately harmful to Bembidion lampros (EU classes).

glyphosate-isopropylammonium

Fish LC_{50} (96 h) for trout and bluegill sunfish >1000, fathead minnows 97, channel catfish 720 mg/l. **Daphnia** LC_{50} (48 h) 930 mg/l. **Algae** E_bC_{50} (72 h) for *Scenedesmus subspicatus* 79.9 mg/l, E_rC_{50} (72 h) 166 mg/l. **Other aquatic spp.** EC_{50} (48 h) for midge larvae 5600, *Litoria moorei* tadpoles >343 mg/l. **Worms** LC_{50} (14 d) for *Eisenia fetida* >5000 mg/kg soil.

glyphosate-potassium

Birds Acute oral LD_{50} for bobwhite quail >2241 mg glyphosate/kg. **Fish** LC_{50} (96 h) for trout >1227 mg glyphosate/l. **Daphnia** LC_{50} (48 h) >1227 mg glyphosate/l. **Algae** E_bC_{50} (72 h) for

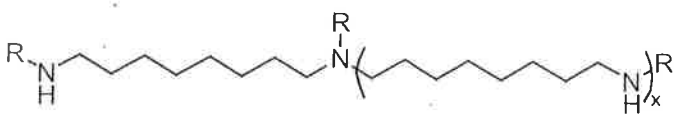
Selenastrum capricornutum 35, E_rC_{50} 54 mg glyphosate/l. **Bees (LD_{50}, µg/bee)** >100 (oral and contact) (48 h)

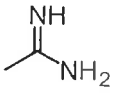
ENVIRONMENTAL FATE: **Animals** In mammals, following oral administration, glyphosate is very rapidly excreted unchanged and does not bioaccumulate. **Soil/Environment** In soil (field), DT_{50} 1–130 d, depending on edaphic and climatic conditions. In water, DT_{50} varies from a few to 91 d. Photodegradation in natural water occurs, DT_{50} 33–77 d; no substantial photodegradation in soil was recorded over 31 d. In a lab. whole system with water and sediment, DT_{50} 27–146 d (aerobic), 14–22 d (anaerobic). The major metabolite in soil and water is aminomethylphosphonic acid. **Plants** Slowly metabolised to aminomethylphosphonic acid (*[1066–51–9]*), which is the major plant metabolite.

404 guazatine

Fungicide

Target Site Multi-site inhibition
FRAC M7, M *guanidine*



R = H or 

NOMENCLATURE:

guazatine
Common name guazatine (BSI, E-ISO, *(f)* F-ISO); guanoctine (former name, BSI)
Chemical Abstracts name guazatine
CAS RN *[108173-90-6]*

guazatine acetates
Chemical Abstracts name guazatine acetate (1:?)
CAS RN *[115044-19-4]*
Other names GTA **EPA PC** 128881 **Development codes** MC 25 (Murphy); EM 379 (Evans)

PHYSICAL CHEMISTRY:

guazatine
Composition The approved common name guazatine was originally defined as applying to 1,1′-iminodi(octamethylene)diguanidine (BSI used the name *guanoctine* from 1970–1972). It is now known that the material marketed commercially is a reaction mixture. Produced by the amidination of tech. iminodi(octamethylene)diamine, commercial guazatine contains numerous guanidines and polyamines; many of these bases are fungicidal. A replacement common name, iminoctadine (*q.v.*), has been established for 1,1′-iminodi(octamethylene)diguanidine.

guazatine acetates
Physical form Yellow to brownish liquid (tech.). **V.p. (mPa)** <0.01
Henry (Pa m^3 mol^{-1}, calc.) 3.8×10^{-8} **log K_{ow}** −1.2 (pH 3); −0.9 (pH 10)

Food and Agriculture Organization of the United Nations

World Health Organization

JOINT FAO/WHO MEETING ON PESTICIDE RESIDUES

Geneva, 9–13 May 2016

SUMMARY REPORT

Issued 16 May 2016

Edited versions of these evaluations and general considerations will be published in the report of the May 2016 JMPR. They are reproduced here so that the information can be disseminated quickly. These drafts are subject to technical editing.

A Joint Meeting of the Food and Agriculture Organization of the United Nations (FAO) Panel of Experts on Pesticide Residues in Food and the Environment and the World Health Organization (WHO) Core Assessment Group on Pesticide Residues (JMPR) was held at WHO Headquarters, Geneva (Switzerland), from 9 to 13 May 2016. Diazinon, glyphosate and malathion were placed on the agenda by the JMPR Secretariat, based on the recommendation of the last session of JMPR to re-evaluate these compounds given the number of new studies that had become available since their last full assessments.

The following extracts of the results of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) are provided to make them accessible to interested parties at an early date.

More information on the work of the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) is available at:

http://www.fao.org/agriculture/crops/thematic-sitemap/theme/pests/jmpr/jmpr-rep/en/

http://www.who.int/foodsafety/areas_work/chemical-risks/jmpr/en/

1. *Evaluation of data for acceptable daily intake (ADI) and acute reference dose (ARfD) for humans*

1.1 Diazinon (22)

Diazinon is an insecticide with a wide range of insecticidal activity. Several epidemiological studies on cancer outcomes following occupational exposure to diazinon were available. The review of these studies provided no convincing evidence of a positive association between exposure to diazinon and non-Hodgkin lymphoma (NHL), but there was weak evidence of a positive association between leukaemia and exposure to diazinon and between lung cancer and exposure to diazinon from one large cohort study only. In studies submitted, diazinon was tested for genotoxicity in an adequate range of assays, both in vitro and in vivo. Overall, these studies provided no convincing evidence of genotoxic effects, and the Meeting concluded that diazinon was unlikely to be genotoxic. The Meeting concluded that diazinon is unlikely to pose a carcinogenic risk to humans from exposure through the diet. After considering all previously evaluated data and the new studies, the Meeting established an ADI of 0–0.003 mg/kg body weight, based on inhibition of acetylcholinesterase activity as the most sensitive end-point. The Meeting reaffirmed the ARfD of 0.03 mg/kg body weight established by the 2006 JMPR based on acute (neuro)toxicity in rats.

1.2 Glyphosate (158)

Glyphosate is a broad-spectrum systemic herbicide. Several epidemiological studies on cancer outcomes following occupational exposure to glyphosate were available. The evaluation of these studies focused on the occurrence of NHL. Overall, there is some evidence of a positive association between glyphosate exposure and risk of NHL from the case–control studies and the overall meta-analysis. However, it is notable that the only large cohort study of high quality found no evidence of an association at any exposure level. Glyphosate has been extensively tested for genotoxic effects using a variety of tests in a wide range of organisms. The overall weight of evidence indicates that administration of glyphosate and its formulation products at doses as high as 2000 mg/kg body weight by the oral route, the route most relevant to human dietary exposure, was not associated with genotoxic effects in an overwhelming majority of studies conducted in mammals, a model considered to be appropriate for assessing genotoxic risks to humans. The Meeting concluded that glyphosate is unlikely to be genotoxic at anticipated dietary exposures. Several carcinogenicity studies in mice and rats are available. The Meeting concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses. In view of the absence of carcinogenic potential in rodents at human-relevant doses and the absence of genotoxicity by the oral route in mammals, and considering the epidemiological evidence from occupational exposures, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet. The Meeting reaffirmed the group ADI for the sum of glyphosate and its metabolites of 0–1 mg/kg body weight on the basis of effects on the salivary gland. The Meeting concluded that it was not necessary to establish an ARfD for glyphosate or its metabolites in view of its low acute toxicity.

1.3 Malathion (49)

Malathion is an insecticide used to control insects on agricultural crops and stored commodities and for vector control. Several epidemiological studies on cancer outcomes in relation to occupational exposure to malathion were available. Overall, there is some very weak evidence of a positive association between malathion exposure and NHL; however, it is notable that the only large cohort study of high quality found no evidence of an association at any exposure level. The evidence is suggestive of a positive association between occupational exposure to malathion and risk of aggressive prostate cancer; however, the evidence base is limited to the one large cohort study. The Meeting concluded that there is some evidence that malathion is carcinogenic in rats and mice. However, the formation of nasal adenomas was due to a local irritancy caused by prolonged exposure to high concentrations of malathion absorbed via inhaled food particles. Scenarios of prolonged, direct and excessive exposure of human nasal tissue to malathion or malathion metabolites following ingestion of residues is unlikely, and therefore these tumours would not occur in humans following exposure to malathion in the diet. Malathion has been extensively tested for genotoxicity, including studies in exposed workers. The Meeting noted that there are numerous reports that malathion can induce oxidative damage in cells, and these results suggest that the observed genotoxic effects occur secondary to the formation of reactive oxygen species, which will exhibit a threshold. Based on consideration of the results of animal bioassays, genotoxicity assays and epidemiological data, the Meeting concluded that malathion and its metabolites are unlikely to pose a carcinogenic risk to humans from exposure via the diet. The current Meeting reaffirmed the ADI of 0–0.3 mg/kg body weight. The margins of exposure between this ADI and the doses causing cancer in mice and rats are 5000-fold and 1200-fold, respectively. The current Meeting also reaffirmed the ARfD of 2 mg/kg body weight. The Meeting concluded that the metabolite malaoxon is approximately 30-fold more toxic than malathion. On this basis, a 30-fold potency factor should be applied to the residue levels for use in both the acute and chronic dietary exposure estimates for malaoxon, and these should be added to the dietary exposures for malathion and compared with the ARfD and ADI for malathion, respectively.

2. General considerations

2.1 General considerations on the evaluation of genotoxicity studies

A large number of genotoxicity studies were evaluated during the present meeting. These were identified through direct submission to JMPR, searches of the publicly available literature and requests to the International Agency for Research on Cancer (IARC) Monographs Secretariat and industry groups. The studies evaluated included unpublished (primarily guideline) studies submitted to support pesticide registration as well as peer-reviewed studies published in the scientific literature. The number, quality and relevance of studies differed widely for each chemical and necessitated that a somewhat different approach be used to evaluate each pesticide. As a general strategy, the studies were separated into categories based largely on phylogenetic relevance and significance of the genetic

end-point measured. The categories used were human biomonitoring, in vivo mammals, in vitro mammalian cells, in vitro bacteria, phylogenetically distant organisms, metabolites in vivo and metabolites in vitro. The evaluation was conducted for the pesticide active ingredient, its formulation products and prominent metabolites, as data were available. For the three pesticides evaluated, the human biomonitoring studies were most often confounded by exposures to other pesticides or considered to have other limitations. Among the genotoxicity studies, in vivo studies in mammals were given the greatest weight, compared with cell culture studies or investigations in phylogenetically distant organisms. Studies of gene mutations and chromosomal alterations were also given more weight than studies measuring other less serious or transient types of genotoxic damage. With regard to route of exposure, studies in which chemicals were administered by the oral route were considered to be of most relevance for evaluating low-level dietary exposures.

Following an evaluation and weighting of the studies, taking the criteria described above and the quality of the studies into account, an overall weight of evidence approach was used to reach conclusions about the genotoxicity of the individual pesticides. An important aspect of the evaluation was whether the genotoxic effect would be likely to occur in humans exposed to low levels of the pesticide present as residues in food.

The Meeting recommended that a guidance document be developed for the evaluation of genotoxicity studies, taking the experience gained from this meeting into account.

2.2 Methods for the evaluation of epidemiological evidence for risk assessment

Identification of compound/cancer sites and screening of papers

There is a large body of literature regarding pesticide exposures and non-cancer outcomes (neurodevelopmental, neurodegenerative and reproductive outcomes, among other health outcomes), but the assessment of the epidemiological evidence on diazinon, glyphosate and malathion was restricted to studies of cancer outcomes. This restriction was partly driven by feasibility reasons: a clinically relevant adverse effect size (or an acceptable level of risk) for a non-cancer outcome must be defined, and the methodologies for hazard identification and characterization based on observational epidemiological findings of non-carcinogenic adverse effects are less well established than those for cancer.

The IARC Monographs on malathion, diazinon and glyphosate referred to a total of 45 epidemiological studies. Databases were searched for any relevant articles published after the studies cited in these Monographs using the following search terms: [(diazinon OR glyphosate OR malathion) AND cancer] and [(diazinon OR glyphosate OR malathion) AND (NHL OR lymphoma OR leukemia OR "lung cancer" OR "prostate cancer")] in PubMed (limited to Humans; published in the last 5 years) and Scopus (limited to 2014–2016). Two studies published since the publication of the IARC Monographs that evaluated at least one of malathion, diazinon or glyphosate were identified in

relation to cancer outcomes. An additional study on prostate cancer, which was not included in the IARC Monographs, was also identified.

The pre-agreed evaluation process shown in Fig. 1 was used to (1) select compound/cancer site combinations to include in this evaluation; (2) screen papers for inclusion/exclusion in this evaluation (Tier 1 screening criteria); and (3) evaluate the information available for risk assessment. In this process, it was noted that there were stand-alone analyses for specific subtypes of non-Hodgkin lymphoma (NHL). The risk for subtypes of NHL was not evaluated separately, as there was insufficient evidence (too few studies or small numbers of cases); the risk for other haematopoietic and lymphoid tumours was also not evaluated separately, as the positive associations identified by IARC were for total NHL.

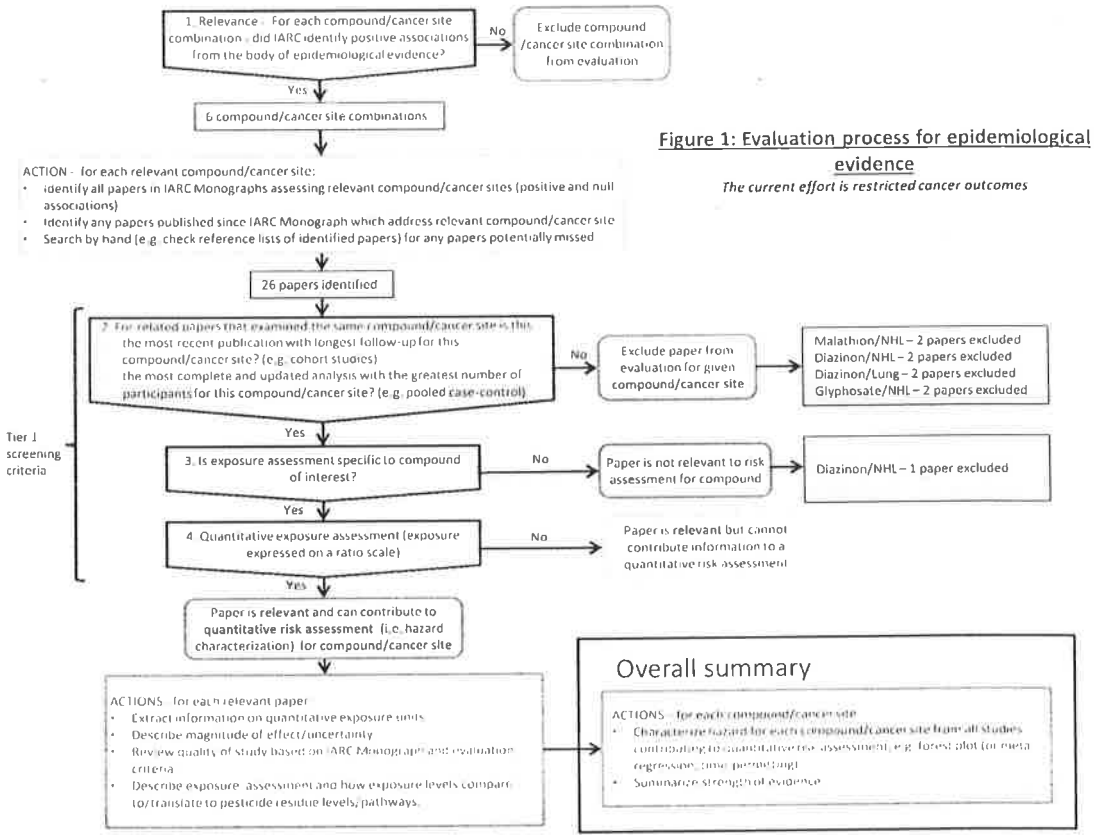


Figure 1: Evaluation process for epidemiological evidence

The current effort is restricted cancer outcomes

Evaluation of evidence for the compound/cancer site associations

Several aspects of each study and of all studies combined were considered in this evaluation, including factors that decrease the level of confidence in the body of evidence, such as risk of bias, unexplained inconsistency and imprecision; and factors that increase the level of confidence, such as large magnitude of effect, dose–response and consistency. The findings for each study were

summarized in tables, and risk estimates for non-quantitative exposure assessment (predominantly ever versus never use) were summarized in forest plots.

Evaluation of information available for risk assessment/hazard characterization

To evaluate overall evidence for dose–response relationships, risk estimates were plotted against quantitative exposure measures (for studies that had used these). The most commonly used quantitative exposure metric was days of use per year. Where studies had used other quantitative exposure metrics (e.g. lifetime days of exposure), data were requested from the authors on median "days of use per year" for the participants in each of the original exposure categories, although this information was not always forthcoming. These additional data allowed the translation and plotting of risk estimates from different studies on the same exposure scale (days of use per year).

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REVIEW ARTICLE

Glyphosate toxicity and carcinogenicity: a review of the scientific basis of the European Union assessment and its differences with IARC

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Abstract Glyphosate is the most widely used herbicide worldwide. It is a broad spectrum herbicide and its agricultural uses increased considerably after the development of glyphosate-resistant genetically modified (GM) varieties. Since glyphosate was introduced in 1974, all regulatory assessments have established that glyphosate has low hazard potential to mammals, however, the International Agency for Research on Cancer (IARC) concluded in March 2015 that it is probably carcinogenic. The IARC conclusion was not confirmed by the EU assessment or the recent joint WHO/FAO evaluation, both using additional evidence. Glyphosate is not the first topic of disagreement between IARC and regulatory evaluations, but has received greater attention. This review presents the scientific basis of the glyphosate health assessment conducted within the European Union (EU) renewal process, and explains the differences in the carcinogenicity assessment with IARC. Use of different data sets, particularly on long-term toxicity/carcinogenicity in rodents, could partially explain the divergent views; but methodological differences in the evaluation of the available evidence have been identified. The EU assessment did not identify a carcinogenicity hazard, revised the toxicological profile proposing new toxicological reference values, and conducted a risk assessment for some representatives uses. Two complementary exposure assessments, human-biomonitoring and food-residues-monitoring, suggests that actual exposure levels are below these reference values and do not represent a public concern.

Keywords Glyphosate · Toxicity · Carcinogenicity · IARC · EFSA · Public health · Consumer risk

Introduction

Glyphosate is the most widely used herbicide in the world. A broad spectrum herbicide, its uses include weed control in agriculture, vegetation control in non-agricultural areas, and harvesting aid as crop desiccant. Its use in agriculture has increased considerably due to the development of glyphosate-resistant GM crop varieties; the herbicide has also been used to control illegal crops through massive aerial applications (Solomon et al. 2007). The widespread use and public debate regarding these uses have aroused societal concern and a scientific controversy on the toxicity of glyphosate (Faria 2015) beyond the scientific debate (Blaylock 2015).

Glyphosate was considered an advantageous herbicide until its use led to the evolution of glyphosate-resistant weeds (Duke and Powles 2008) and studies suggesting effects of glyphosate-based formulations in humans and wildlife were published. Interest in glyphosate has increased exponentially among scientists, and the subject accounted for 5% of the articles on pesticides included in PubMed during 2015. About 25% of the articles cover the toxicity endpoints in humans and all types of organisms, and the majority is conducted with glyphosate-based formulations, containing other ingredients. Some

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ingredients may be more toxic than glyphosate for non-plant species (Kim et al. 2013; Mesnage et al. 2013; Nobels et al. 2011), ingredients classified as carcinogenic or mutagenic are not expected to be used and must be indicated in the label, however, the full composition of the formulation is not disclosed by the manufacturers, therefore, it is impossible for researchers to apply mixture toxicity methods and attribute toxicity to specific ingredients.

The risk assessment of a pesticide for human health integrates two aspects. First, the hazard identification clarifies the toxicological profile of the substance, setting the type of health effects it is expected to produce in humans depending on the level of exposure, triggering the hazard classification and setting the toxicological reference values to be used in the risk assessment. Then, for each intended use, the expected level of exposure is calculated and compared with the reference values. While the hazard potential is intrinsic and, therefore, expected to be equivalent in all evaluations, the risk is related to the use of the substance—which is defined as the likelihood and magnitude of adverse effects—and strongly depends on the patterns and conditions of use.

Glyphosate has been the subject of regular assessments by national and international regulatory agencies (JMPR 2006; Williams et al. 2000). All had established that glyphosate has a relatively low toxicity in mammals. However, a recent report from the International Agency for Research on Cancer (IARC) concluded that the herbicide and its formulated products are probably carcinogenic in humans (Guyton et al. 2015a, b; IARC 2015). The aim of IARC's assessments is to identify carcinogenicity hazards as a first step in carcinogenic risk assessment. IARC assessments do not include recommendations regarding regulatory or legislative decisions; they are scientific evaluations informing regulatory assessments. Consequently, the IARC conclusion triggered a reconsideration of the evidence on carcinogenicity in the EU evaluation, and more recently by the Joint FAO/WHO Meeting on Pesticide Residues. The European Union renewal process (European Food Safety Authority 2015a, b; Germany 2015) was the first comprehensive regulatory assessment of glyphosate conducted after the IARC evaluation. Following a detailed assessment of all available information, the European assessment reached a different conclusion, increasing the scientific and social debate. In 2016 the Joint FAO/WHO Meeting on Pesticide Residues concluded that glyphosate is not carcinogenic in rats but could not exclude the possibility that it is carcinogenic in mice at very high doses, this information was used in the risk assessment concluding that glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet (JMPR 2016). This manuscript explores possible reasons for the different conclusions, with a focus on the EU assessment, as this is the evaluation in which the authors have been involved.

Typically, regulatory assessments come to conclusions similar to those of IARC, but there are exceptions (Pearce et al. 2015). Scientific divergences may result from different sets of evidence, different approaches and methods, or different interpretations when weighing ambiguous results. Divergences are particularly likely when one evaluation includes additional evidence. In this context, it is important to mention that the EU evaluation, which considered studies not available to IARC, also updated the toxicological profile of glyphosate, proposing new toxicological reference values.

IARC monographs cover carcinogenicity hazard identification. When statistical associations between exposure and cancer incidences are observed in epidemiological studies, the assessment of causal relationships may lead to divergent conclusions (Rhomberg 2015a, b). The comparison of both glyphosate assessments is used below to explain the different aims, methods and possible divergences between regulatory and IARC assessments—focusing on the glyphosate carcinogenicity hazard identification as a case study—and, more importantly, their role in the assessment of risks to consumers and public health concerns. The example is particularly useful as both evaluations were conducted within the same period, and as the EU assessment, based on the United Nations Globally Harmonised System (UN-GHS) for classification of chemicals, is also relevant in the broad international context.

Methodology: scientific assessment of carcinogenicity and its use in the regulatory context

Pesticides are heavily regulated chemicals and require pre-marketing authorisation in most jurisdictions. The EU system also includes a renewal process, requiring all pesticides to be regularly re-assessed in the light of new scientific developments and information requirements. The EFSA assessment (European Food Safety Authority 2015b) followed an evaluation carried out by the European Commission in 2002.

The identification of carcinogenic chemicals and carcinogens in food is of high societal and scientific interest (Barlow and Schlatter 2010). The communication of the outcome of the risk assessment is complex and controversial in the case of equivocal results (Downes and Foster 2015). The identification of a mutagenic or genotoxic mechanism plays an important role in risk assessment and requires a critical evaluation of the data as well as expert judgment (Eastmond 2012). The hazard assessment is linked to the classification; the EU uses the hazard assessment system

for chemicals developed by the United Nations following the 1992 UN Earth Summit (Pratt 2002). This Globally Harmonised System for classifying chemicals replaces previous national and international approaches, is specifically recommended by FAO to be used for pesticides, and is implemented in the EU Classification, labelling and packaging (CLP) regulation—(EC) No 1272/2008—and other jurisdictions (UNECE http://www.unece.org/trans/danger/publi/ghs/implementation_e.html).

IARC and regulatory assessments are usually complementary. The different roles, methods and information sources of IARC and regulatory assessments, as well as the implications for public health, must be considered in case of divergences and are summarised in Table 1. IARC identifies carcinogenic hazards resulting from occupational, environmental, and lifestyle exposures and agents as a first step of the risk assessment process, and has developed an internationally recognised grouping system that includes defined criteria and methodology (Guyton et al. 2015a, b; Lauby-Secretan et al. 2016; Pearce et al. 2015; Straif et al. 2014). The recently developed approach for assessing mechanistic information, based on the characteristics of IARC group 1 carcinogens, was applied for glyphosate (Smith et al. 2016). Regarding data sources, IARC assessments are primarily based on published evidence, i.e. scientific publications and regulatory assessments; industry-sponsored studies are used when reviewed and reported in regulatory evaluations, becoming a relevant secondary source for regulated agents such as pesticides. Both, scientific publications and mandatory industry-sponsored studies, were primary sources in the EU evaluation.

For pesticides, IARC identifies the "carcinogenic agent" as the active pesticide substance and its commercial formulations; the specific role of the other formulation ingredients in the occurrence of effects is not considered separately from the active ingredients. This is in line with the role of human evidence in IARC assessments. Epidemiological studies of farmers and consumers have very limited information on actual exposure levels (Ntzani et al. 2013), and use the pesticide active substance as descriptor, combining individuals exposed to different formulations without discriminating the different compositions. In the regulatory context, each formulation should be assessed according to its composition, identifying the role of the active substance and of the other ingredients; and the risk management measures are set for the chemical responsible for the effect, either active substance or co-formulant.

The UN-GHS and IARC frameworks use different terminology, but the definitions for sufficient and limited evidence in humans and in animals are similar and can be used to establish equivalences between both schemes, as presented in Table 2.

This approach allows a comparison of the pesticides evaluated by IARC with the current EU classification (Table 3 and supplementary material Annex 1). The EU classification includes scientific assessments conducted by the European Chemicals Bureau of the European Commission—some, but not all, based on EFSA evaluations—and by the Committee for Risk Assessment of the European Chemicals Agency.

A total of 53 pesticides have been assessed under both systems. For about half—29 out of 53—the classifications are equivalent; the EU classification is more severe/conservative for 14 pesticides and less severe/conservative for 11. It should be noted that 8 out of the 11 pesticides with more severe/conservative classification by IARC are those

Table 1 Comparison of IARC and regulatory assessments roles and methodological elements

Issue	IARC	EU regulatory assessment
Role	Hazard based identification. First step to be used by authorities in their risk assessments. No regulatory power	Scientific assessment covering hazard identification (classification), hazard characterisation (setting toxicological reference values), exposure assessment, and risk characterisation Formal support for decision making
Coverage	IARC selection, based on criteria such as identified concern or human exposure. Chemical, physical, biological or behavioural "agents" 58 pesticides	Mandatory. 1355 pesticide active substances in the EU data base. Chemical and microbial pesticides
Method	IARC developed methodology, described in the "preamble". Applicable to all agents	For chemical pesticides, hazard identification based on UN GHS criteria Detailed guidance from ECHA available
Sources	Review of published information. Summaries of industry sponsored studies used as secondary source if obtained from regulatory agency reports	Full set of mandatory (OECD guidelines) GLP studies and epidemiological data Review of scientific peer-review publications, last 10 years Information collected through a public consultation
Formulations	"Agent" grouped as active substance and all formulated products together	UN GHS principles applied to the active and then to each formulation, accounting for all other ingredients

Table 2 Proposed equivalences between the UN-GHS and IARC classification schemes

UN-GHS and CLP	*Category 1A* Substances known to have carcinogenic potential for humans Largely based on human evidence	*Category 1B* Substances presumed to have carcinogenic potential for humans Largely based on animal evidence	*Category 2* Substances suspected to have carcinogenic potential for humans Evidence obtained from human and/or animal studies but not sufficiently convincing to place the Substance in Category 1A or 1B	*No classification* No sufficient evidence for classifying the substance as carcinogenic	
IARC	*Group 1* The agent is a carcinogen for humans. This category is only used when sufficient indications of carcinogenicity for humans are available	*Group 2A* The agent is probably carcinogenic for humans. The classification of an agent in this category is recommended if there is no formal evidence of carcinogenicity in humans, but corroborating indicators of its carcinogenicity for humans and sufficient evidence of carcinogenicity in experimental animals	*Group 2B* The agent is possibly carcinogenic for humans. There is limited evidence of carcinogenicity in humans and evidence for animals, or insufficient evidence for human beings but sufficient evidence of carcinogenicity in experimental animals	*Group 3* Agent-not classifiable as to its carcinogenicity to humans. (Insufficient evidence for human beings and insufficient or limited for animals)	*Group 4* Agent probably not carcinogenic for humans. (Evidence suggesting lack of carcinogenicity in humans and in experimental animals)

Table 3 Overall comparison of the carcinogenicity assessments of pesticides conducted by EFSA and IARC (see supplementary material for information on the pesticides classified in each category)

	Category 1A	*Category 1B*	*Category 2*	*No classification*		*Not assessed/no data*
EU	0	17	53	30		4
	Group 1	*Group 2A*	*Group 2B*	*Group 3*	*Group 4*	*Not assessed*
IARC	3	8	13	34	0	56

assessed in recent IARC monographs. New substances are evaluated and others re-evaluated regularly, leading to changes in the classification; thus the table represents just a "screen-shot" of two rolling processes. Differences with IARC and between jurisdictions have also been reported for other regulatory assessments (Choi and Lim 2010). Both IARC and regulatory classifications are based on the information available at the time of the evaluation. For pesticides, the identification of possible concerns triggers the generation of additional evidence and a subsequent evaluation, consequently, some differences are not real scientific divergences but the result of expert re-evaluations based on different sources of evidence. This may have played a role in the case of glyphosate, as discussed below.

Discussion

Understanding the divergence: glyphosate carcinogenicity assessment

The carcinogenicity of glyphosate has been reviewed by several national and international agencies (Ibrahim 2015). The outcome of the EU assessment, the differences with the IARC evaluation (IARC 2015), and the authors' views explaining these differences, are summarised below. Additional details are provided in the supporting information.

Human evidence

IARC (2015) offered the most up-to-date review of human epidemiological studies on glyphosate. Positive evidence regarding an association between exposure to glyphosate and non-Hodgkin lymphoma, observed in some case-control studies but not confirmed by cohort studies, was considered sufficient by IARC to conclude on "limited evidence" in humans. Limited evidence is defined as a positive association observed between exposure to the agent and cancer, for which a causal interpretation is considered to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence. This definition was developed by IARC and introduced in the UN-GHS criteria (United Nations 2003) and EU Regulation (EC) No 1272/2008. EFSA re-assessed the same information; the association with non-Hodgkin lymphoma was discussed during an expert meeting. The statistically significant association was considered limited due to low power, lack of consistency, and the view that greater weight should be given to the cohort study for non-rare tumours. Considering causality, the majority of the experts concluded that the epidemiological evidence was very limited, and insufficient for classification. Although the role of the weight attributed to case–control studies versus cohort studies cannot be fully ruled out, the main reason for the divergent views could be the possibility of bias, chance results and confounding effects, as IARC concluded that the limited evidence in humans was supported by sufficient evidence of carcinogenic potential in animals and strong mechanistic evidence for genotoxicity and oxidative stress. As explained below, the EU evaluation used additional evidence regarding animal carcinogenicity and genotoxicity, and reached different conclusions.

Carcinogenicity in animals

Information sources There is only one published study on the carcinogenicity of the active substance glyphosate in rats (Chruscielska et al. 2000), which showed no significant increase in tumour incidences in any treated group. Two additional published studies on glyphosate formulations, the first one on initiation-promotion in mice (George et al. 2010) and the second one, a study of rats (Seralini et al. 2014) that was retracted and republished creating some controversies (Fagan et al. 2015), were considered inadequate by IARC and EFSA for carcinogenicity assessment (European Food Safety Authority 2012; IARC 2015). Consequently, industry-sponsored studies, required by several jurisdictions worldwide, have constituted the basis for the assessment of animal carcinogenicity by both IARC and EFSA. As expected for a regulatory assessment, EFSA assessed the original study reports. According to their principles, IARC used unpublished studies based on secondary sources, i.e. the information on the studies as published by JMPR (2004) and US-EPA (1993). The time difference, over a decade, between the IARC monograph and the published regulatory assessments must be considered. Five new studies, not assessed by the JMPR and US-EPA, and therefore, not considered by IARC, were considered valid and included in the

EU assessment. The IARC assessment is based on the re-assessment of industry-sponsored studies, two in mice and four studies in rats, plus the negative published study in rats. The EU assessment included five additional valid studies, two in mice and three in rats; one mouse study was excluded due to a likely viral infection in the experimental population and one rat study was considered inadequate due to study deficiencies. Table 4 summarises the studies used in the EU assessment; additional information is provided in Table S-2 as supplementary material, with links to the detailed summaries for each study and its assessment as published in the EFSA background document (Germany 2015). Additional information and raw data have been published as supplementary information in a recent industry-sponsored review of glyphosate carcinogenicity (Greim et al. 2015).

Assessment of the available evidence In its weight of evidence, the IARC Working Group considered a statistically significant trend for renal tumours in male mice in one study (study A in Tables 4, 5) and for haemangiosarcoma in the other (study B in Tables 4, 5). No statistically significant increase in tumour incidence in females was observed in these studies. In the weight of evidence in rats, the IARC Working Group considered increases in the incidence of adenomas, with no evidence of progression to carcinomas, in pancreatic islet cells in males (studies E and F in Table 4), hepatic cells in males (study E in Table 4) and thyroid C-cell in females (study E in Table 4). No increase in tumour incidence was observed in three studies (studies G, K and M in Table 4). The EU assessment followed the weight of evidence approach required by the UN-GHS criteria (United Nations 2015) and further clarified in the ECHA guidance (European Chemicals Agency 2015). The statistical significance found in trend analysis in some studies was balanced against the lack of statistical significance in pair-wise comparison tests, lack of consistency in multiple animal studies, slightly increased incidences only at dose levels at or above the Maximum Tolerable Dose (MTD), lack of pre-neoplastic lesions and/or whether the studies fell within the relevant historical control range. A specific comparison of tumour incidences in male CD-1 mice from four carcinogenicity studies (no change in tumour incidence was observed in females) is provided in Table 5, and the detailed scientific assessment and weight of evidence for each tumour type is summarised in Table 6.

Comparison of both weight of evidence approaches As indicated by Portier et al. (Portier et al. 2014), individual scientific studies are rarely, if ever, conclusive. In our view, this is particularly relevant when assessing the carcinogenicity potential in humans using animal studies, and supports the need for a consistency check combining all available studies as mandated in the UN-GHS criteria.

In the absence of conclusive human evidence, and despite some views suggesting the need for re-assessing its relevance (Beyer et al. 2011; Marone et al. 2014; Osimitz et al. 2013), rodent long-term toxicity/carcinogenicity studies are used for predicting carcinogenicity in humans (Doktorova et al. 2012). False positives and false negatives should both be considered, weighing the evidence (Lutter et al. 2015; Rhomberg 2015a, b; Rhomberg et al. 2013) and assessing specifically human relevance; and linked to the MTD concept, the relevance of toxicity-induced carcinogenic effects observed in experimental animals only at very high doses. The UN-GHS, and therefore, the EU CLP approach are based on UN harmonised criteria for weighing the evidence from rodent studies. Regulatory (European Chemicals Agency 2015) and non-regulatory (McGregor et al. 2010) guidance is available for weighing the evidence in line with the UN-GHS criteria. Table 7 summarises the assessment of the different UN-GHS Weight of Evidence elements in the EU assessment, and includes a comparison with the weight provided in the IARC evaluation. It should be noted that the authors of this paper did not participate in the IARC assessment, and therefore, the IARC columns are based on the information extracted from the IARC preamble and monograph, and do not reflect the Working Group discussions except when specifically reported in the monograph. The elements detailed in Tables 5, 6 and 7, and used in the EU evaluation, are not only specific components of the regulatory guidance (European Chemicals Agency 2015), but, as described below, are also fully supported by current scientific knowledge on the assessment of animal studies.

Due to the large number of studies, the assessment of chance results is particularly relevant. Dose–response within the study, consistency among similar studies, consistency or justified differences between sexes, and comparison with historical controls, are considered key elements for identifying chance effects. The Bradford Hill guidelines published in 1965 are still considered a reference for assessing causality (Wakeford 2015), and have been included in the IPCS framework and its respective updates (Boobis et al. 2006, 2008; Meek et al. 2014a; Sonich-Mullin et al. 2001). Although the framework focuses on the relevance of the mode of action, dose–response relationships and consistency among studies are also indicated as key elements. The statistical assessment is the first step for assessing the results of the toxicity tests, and has received significant attention from both, regulatory bodies (e.g. OECD guidelines on testing and assessments of chemicals) and academics (Hothorn 2014); nevertheless, the statistical analysis should be considered part of an overall assessment. This is particularly relevant in cases such as glyphosate, where the statistical analysis is inconsistent or inconclusive, with significant

Table 4 Review of long-term chronic toxicity and carcinogenicity studies considered during the EU assessment

Study reference—Authors	Duration, strain, study type	Dose levels (NOAEL/LOAEL) mg/kg bw per day	Critical effect at the LOAEL
Mice long-term chronic toxicity and carcinogenicity studies used in the EU evaluation			
A - Knezevich and Hogan (1983)	2 year, CD-1, OECD TG 451/453	0, 157, 814, 4841 (157/814)	Males: body weight reduction, hepatocellular centrilobular hypertrophy and bladder epithelial hyperplasia
B - Atkinson et al. (1993)	2 year, CD-1, OECD TG 451	0, 100, 300, 1000 (1000/>1000)	Equivocal enlarged/firm thymus, not associated with histopathological findings (considered not biologically relevant)
C - Sugimoto (1997)	18 month, CD-1 (ICR), OECD TG 451	0, 153, 787, 4116 (153/787)	Body weight gain, reduction food consumption and efficiency, loose stool, caecum distended and increased weight, prolapse and anus ulceration
D - Wood et al. (2009)	18 month, CD-1 (ICR), OECD TG 451	0, 71, 234, 810 (810/>810)	No adverse effects observed
Rat long-term chronic toxicity and carcinogenicity studies used in the EU evaluation			
E - Lankas (1981)	26 month, Sprague–Dawley rat, combined chronic toxicity/carcinogenicity;, Not Good Laboratory Practice (GLP) compliant	0, 3, 10.3, 31.5 (31.5/>31.5)	No adverse effects observed*
F - Stout and Ruecker (1990)	2 year, Sprague–Dawley rat, US-EPA F 83 – 5	0, 89, 362, 940 (89/362)	Reduction body weight and gain, increase liver weight, stomach mucosal inflammation, cataracts, decrease urine pH, survival <50% in all groups incl. controls
G - Atkinson et al. (1993)	2 year, Sprague–Dawley rat, US-EPA F 83 – 5	0, 10, 100, 300, 1000 (100/300)	Pronounced salivary gland findings, increase AP and liver weight
H - Suresh (1996)	2 year, Wistar rat, OECD TG 453	0, 6.3, 59.4, 595.2 (60/595.2)	Cataracts, increase AP
I - Lankas 1997	12 month, Wistar rat, OECD TG 452	0, 141, 560, 1409 (141/560)	Reduction in body weight, food cons and utilization, increase AP, focal basophilia of acinar cells of parotid salivary gland (not weighed)
J - Enomoto (1997)	2 year, Sprague–Dawley rat, OECD TG 453	0, 104, 354, 1127 (104/354)	Reduction body weight, gain, food cons (initially) and utilization, increase loose stool, increase tail masses due to follicular hyperkeratosis and abscesses, caecum: distension and increase weight, pH reduction and dark appearance of urine
K - Brammer (2001)	2 year, Wistar rat, OECD TG 453	0, 121, 361, 1214 (361/1214)	Reduction body weight, food cons and (initially) utilization, clinical chemistry findings (increase AP and ALAT activity and bilirubin, decrease urine pH), kidney papillary necrosis, prostatic and periodontal inflammation

Arch Toxicol (2017) 91:2723–2743

Table 4 (continued)

Study reference—Authors	Duration, strain, study type	Dose levels (NOAEL/LOAEL) mg/kg bw per day	Critical effect at the LOAEL
L - Wood et al. (2009)	2 year, Wistar rat. OECD TD 453	0. 86. 285. 1077 (285/1077)	Reduction body weight gain, transient increase AP. changes in distribution of renal mineralisation, increase adipose infiltration of bone marrow (indicative of hypoplasia)
M - Chruzielska et al., 2000	24 month, Wistar rat, in drinking water	0. 300, 900 or 2700 mg/L	No significant increase in tumour incidence
Industry-sponsored GLP studies considered non-acceptable during the EU assessment			
N - Kumar (2001)**	18 month, Swiss albino mice. OECD TG 451	Title: Carcinogenicity Study with Glyphosate Technical in Swiss Albino Mice	
O - Bhide (1997)***	2 year Sprague–Dawley rat, OECD TG 453	Title: Combined Chronic Toxicity/Carcinogenicity Study of Glyphosate Technical in Sprague Dawley Rat	
Published studies conducted with glyphosate-based formulations and considered non-reliable for the assessment of glyphosate carcinogenicity during the EU assessment			
P - George et al. (2010 carcinogenicity)	Non-guideline mechanistic study conducted with topical application of glyphosate-based formulation	Title: Studies on glyphosate-induced carcinogenicity in mouse skin: A proteomic approach	
Q - Seralini et al. (2012). re-published 2014	24-month study (10 males and 10 females per group) Sprague–Dawley rats in drinking water	Title: Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize	

*The dose levels used in this study are too low and the study is not considered adequate to assess glyphosate chronic toxicity/carcinogenicity

**Study N found unreliable after detailed assessment, due to the occurrence of viral infection in all groups including controls

***Study O was considered not acceptable because no core information on the test substance such as batch number or purity was given and, thus, it is not clear what was in fact tested. Furthermore, the study presented many deficiencies

Table 5 Summary of selected tumour incidences in male CD-1 mice from four studies with glyphosate and historical control data

Dose range	Tumour incidence/number of animals examined												
	Control group				Low dose			Intermediate dose		High dose			Very high dose***
Dose (mg/kg bw per day)	0	0	0	0	71	100	157	165	234	300	810	814	838	1000	4348	4841
Study ID	A	B	C	D	D	B	A	C	D	B	D	A	C	B	C	A
Study duration (months)	24	24	18	18	18	24	24	18	18	24	18	24	18	24	18	24
Survival	20/50	26/50	26/50	39/51	41/51	25/50	16/50	34/50	39/51	29/50	35/51	17/50	27/50	25/50	29/50	26/50
Renal tumours[#]	1/49	2/50	0/50	0/51	0/51	2/50	0/49	0/50	0/51	0/50	0/51	1/50	0/50	0/50	2/50	3/50
Malignant lymphoma*	2/48	4/50	**2/50**	0/51	1/51	2/50	5/49	**2/50**	2/51	1/50	5/51	4/50	**0/50**	6/50	**6/50**	2/49
Haemangiosarcoma**	0/48	**0/50**	0/50	2/51	1/51	**0/50**	0/49	0/50	2/51	**0/50**	1/51	1/50	0/50	**4/50**	2/50	0/49

Study ID: A = TOX9552381 (1983), PWG re-evaluation, B = TOX9552382 (1993), C = ASB2012-11493 (1997); D = ASB2012-11492 (2009)

*Study A: Malign lymphoblastic tumours (3 categories) instead of malignant lymphoma which was not mentioned as a pathological entity

**Whole body/multiple organ

***Dosage exceeded the OECD-recommended limit dose of 1000 mg/kg bw/day and the MTD

Numbers in bold refer to values within acceptable HCD; no HCD is available for the other values (not bold) and no exceedance of HCD was recorded in mice treated with glyphosate

[#]Renal tumours: combined incidence of adenoma and carcinoma

differences in the trend, but not in the pair-wise analysis. Lack of consistency at similar doses in the same species and strain and lack of dose–response relationships can be observed for malignant lymphomas in mice (Tables 5, 6) and adenomas in rat (Table 6). Kobayashi et al. (2010) reviewed the grounds for considering statistically significant changes as incidental, observing similar trends for unpublished and peer-reviewed scientific publications. Lack of dose–response is reported as the main justification for disregarding the results as incidental, followed by lack of physiological/toxicological significance of the effects and the comparison with historical controls. These studies support the concern surrounding conclusions that are based only on statistical significance of increased tumour incidences in a particular study, without considerations of the biological relevance of the finding.

Although the concurrent control group is always the most relevant comparator, the use of historical control data, also in combination with background incidental lesions (McInnes and Scudamore 2014), can be essential in cases of equivocal results to detect both, false positive and false negative situations. In addition to best practices (Greim et al. 2003; Keenan et al. 2009), graphical visualisations (Elmore and Peddada 2009) and statistical approaches (Dinse and Peddada 2011; Peddada et al. 2007) have been developed, although direct comparison with the historical control range in the test laboratory around the time of the study is the approach mostly used in the regulatory context, and preferred in the EU assessment. This approach was considered for malignant lymphomas and haemangiosarcomas in mice when the studies reported the historical range for the test laboratory.

Excessive toxicity, for instance toxicity at doses exceeding the MTD, can cause effects such as cell death (necrosis) with associated regenerative hyperplasia, which in turn can lead to tumour development as a secondary effect, unrelated to the intrinsic potential of the substance itself to cause tumours at lower and less toxic doses (European Chemicals Agency 2015; Knight et al. 2006). Also in the assessment of cell proliferation as mode of action for non-genotoxic carcinogens, systemic toxicity and overt cytotoxicity in the target tissue should be avoided (Wood et al. 2015). It has been suggested that almost all chemicals, including those non-genotoxic and without structural alerts for carcinogenicity, would produce statistically significant trends if tested at or above the MTD in a sufficiently large number of animals (Gaylor 2005). Significant trends for tumour induction were observed in two mouse studies but only at very high doses, well above the proposed top dose for carcinogenicity studies (OECD 2012) of 1000 mg/kg bw per day; clear indications of toxicity were observed at these high doses, such as reduced body weight, histopathological changes in the bladder and liver, and other toxic

Table 6 Summary of the weight of evidence of the EU assessment for the different tumour types

Tumour type/species	Significant trends	Weight of evidence in EU assessment
Renal tumours, mice	2 out of 4 studies TOX9552381 (6% combined adenomas and carcinomas in males at 4841 mg/kg bw day) ASB2012-11493 (4% adenomas in males at 4348 mg/kg bw day)	Both studies, trends observed only at high dose (>4000 mg/kg bw per day), where general toxicity (such as reduced bw, histopathological findings in liver, and bladder in one study and reduced bw gain, severe gastro-intestinal effects in the other) may be confounding factors No statistical significance in a pair-wise comparison One trend in one study did not consider the higher survival at the top dose
Malignant lymphomas, mice	2 out of 4 studies ASB2012-11493 (12% males at 4348 mg/kg bw day) ASB2012-11492 (10% males at 810 mg/kg bw day)	Malignant lymphomas is one of the most common neoplasms in CD-1 mice, females being more prone to this tumour type than males No statistical significance in a pair-wise comparison First study within historical controls and trend observed only at high dose (>4000 mg/kg bw per day), where general toxicity may be a confounding factor Second study inconsistency in results among 4 studies comparing similar dose levels
Haemangiosarcomas, mice	2 out of 4 studies TOX9552382 (8% males at 1000 mg/kg bw day) ASB2012-11493 (4% males at 4348 mg/kg bw day)	No statistical significance in a pair-wise comparison First study within historical control range Second study trend observed only at high dose (>4000 mg/kg bw per day) where general toxicity may be a confounding factor
Hepatocellular adenomas, rats	1 out of 8 studies TOX9300244 (15% males at 940 mg/kg bw day)	No statistical significance in a pair-wise comparison Marginal trends in benign tumours limited to one sex, not reproduced among 8 long term studies (3 studies in SD rats and 5 studies in Wistar rats)
Thyroid C-cell adenomas, rats	1 out of 8 studies TOX9300244 (10% females at 457 and 1183 mg/kg bw day)	No statistical significance in a pair-wise comparison Marginal trends in benign tumours limited to one sex, not reproduced among 8 long term studies (3 studies in SD rats and 5 studies in Wistar rats)
Pancreatic islet cell adenomas, rats	Incidences without dose response trends in 2 out of 8 studies	Lack of dose–response does not support an effect related to glyphosate administration
All other tumours, mice and rats	No increased incidences observed in 4 mice and 8 rat studies	No observed incidences in a large number of valid studies

signs; consequently, the tumour induction trends were considered confounding effects due to excessive toxicity.

Mechanistic assessment

The relevance of the mode of action for humans constitutes the basis of the IPCS framework (Boobis et al. 2006, 2008; Meek et al. 2014a; Sonich-Mullin et al. 2001). Mode of action is defined as a biologically plausible series of key events leading to an effect (Sonich-Mullin et al. 2001) and involves interdependent networks of events with feedback loops. Differences in networks between and within human and animal populations account, in part, for interspecies differences and human variability (Meek et al. 2014a). Current approaches explore the applicability of the Adverse Outcome Pathway approach (Collier et al. 2016; Edwards et al. 2016; Zhou 2015) as a framework for linking the initial molecular interactions with the tumour promotion though plausible key events (Becker et al. 2015; Downes and Foster 2015). As the EU evaluation concluded that the incidences were due to chance and bias and the evidence does not indicate that glyphosate is an animal carcinogen, no further assessment of relevance for humans was required.

IARC, with a different focus, not targeted to individual chemicals but to a broad range of agents, has recently developed a new weight of evidence scheme, by extracting the "key characteristics" from the physical/chemical/biological/behavioural agents classified by IARC in category 1 (Smith et al. 2016). These key characteristics are defined as common properties, not to be considered mechanisms of Adverse Outcome Pathways, although are postulated as a method to synthesize information and develop adverse outcome networks. The ten characteristics are the abilities of an agent to: (1) act as an electrophile either directly or after metabolic activation; (2) be genotoxic; (3) alter DNA

Table 7 Summary of the UN-GHS Weight of Evidence (WoE) elements in the EU assessment and comparison with the weight provided in the IARC assessment

UN-GHS and EU CLP WoE elements	Regulatory guidance (ECHA, 2015) and scientific support	Evaluation method in the IARC Preamble	Relevance for the glyphosate WoE	
			EU assessment	Comments on IARC assessment
(a) Tumour type and background incidence	Relevance for humans, due to the relevance of the mode of action(Meek et al. 2014a, b), or tissues with no human equivalents. Spontaneous incidences and use of historical control data(Dinse and Peddada 2011; Greim et al. 2003; Keenan et al. 2008; Ma et al. 2002; Massarelli et al. 2013)	Relevance for humans, e.g. species-specific mechanism that does not operate in humans The use of historical data is mentioned	All valid studies are considered negative. No need for mode of action evaluation Historical control data from the same laboratory were considered when available	All tumours were assumed relevant for humans No information on the use of historical control data is provided except the consideration of some tumours as "rare"
(b) Multi-site responses	If observed, increases the evidence (Dybing et al. 1997)	Consistency of the results across target organ(s) and spectrum of neoplastic response	No significant incidences observed in the valid studies Consistency among studies was considered	Based on statistically significant trends for different tumour types. Assessment limited to a subset of the available studies
(c) Progression of lesions to malignancy	If observed, increases the evidence	The spectrum of neoplastic response, from preneoplastic lesions and benign tumours to malignant neoplasms	Specifically considered for individual studies	Specifically considered for individual studies
(d) Reduced tumour latency	Only relevant for unusual tumours	Sufficient for considering the agent as carcinogen	Not relevant	No indications are provided
(e) Whether responses are in single or both sexes	A consistent mode of action is required for tumours observed in only one sex	No specific indications for the evaluation of tumours occurring in a single sex are provided	Contributes to the lack of consistency assessment as no sex related mode of action is postulated	All trends were significant only in one sex, but no sex mediated mode of action is discussed
(f) Whether responses are in a single species or several species	If observed in several species increases the evidence	If observed in several species increases the evidence	No significant incidents were identified for mice or rats	Based on positive trends in both mice and rats
(g) Structural similarity to a substance(s) for which there is good evidence of carcinogenicity	Includes SAR, QSAR, read across and grouping	The possibility for using information from structurally similar agents is mentioned	Not relevant, the assessment is based on studies on glyphosate	Not relevant, the assessment is based on studies on glyphosate
(h) Routes of exposure	Includes local tumours	The exposure route should be mentioned	Assessment based on oral studies	Assessment based on oral studies
(i) Comparison of absorption, distribution, metabolism and excretion between test animals and humans	Also relevant for considering the role of metabolites	Comparison should be made when possible	Not relevant	Not relevant
(j) The possibility of a confounding effect of excessive toxicity at test doses	Effects observed only at doses exceeding the maximum tolerable dose should be checked for confounding effects of excessive toxicity	Not mentioned in the preamble. NOAELs and LOAECs for each study are not reported	Considered for tumours in mice	Effects observed only at high doses with excessive toxicity are included in the trend assessment. No additional information is provided

Table 7 (continued)

UN-GHS and EU CLP WoE elements	Regulatory guidance (ECHA, 2015) and scientific support	Evaluation method in the IARC Preamble	Relevance for the glyphosate WoE	
			EU assessment	Comments on IARC assessment
(k) Mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity	The IPCS framework and related approaches (Boobis et al. 2006, 2008; Meek et al. 2014a; Sonich-Mullin et al. 2001) offers general guidance. IARC (1994, 1999) and ECHA (2015) list specific tumours considered not relevant for humans. Mutagenicity and genotoxicity play a key role in the assessment and in particular the assessment of non-threshold genotoxic-carcinogenic modes of action	The possible mechanism should be identified when possible. The assessment of genotoxicity is described in the preamble, *in vivo* data on humans and mammals have preference. No mention to the "ten key characteristic approach" is included in the preamble	The genotoxicity assessment is based on mammalian studies, and concluded as negative for glyphosate, as all studies are negative except at very high doses with confounding cytotoxicity. Genotoxicity of a co-formulant and of some glyphosate formulations cannot be ruled out, and should be addressed	The conclusion of strong evidence on genotoxicity and oxidative stress for glyphosate and glyphosate based formulations is one of the key arguments of the IARC proposal. The differences between glyphosate and glyphosate based formulations reported in several studies are presented but no further discussed

repair or cause genomic instability; (4) induce epigenetic alterations; (5) induce oxidative stress; (6) induce chronic inflammation; (7) be immunosuppressive; (8) modulate receptor-mediated effects; (9) cause immortalization; and (10) alter cell proliferation, cell death, or nutrient supply. It should be noted that this new approach has been applied to the recent IARC monographs, including the assessment of glyphosate.

Genotoxicity

The EU evaluation considers *in vitro* genotoxicity tests and *in vivo* studies performed in mammals, as those are considered to be more relevant for the assessment of the risk to humans (Yauk et al. 2015). Sixteen *in vivo* studies in somatic cells and two *in vivo* studies on germ cells were reported on rodents orally treated with dose levels up to 5000 mg/kg bw, or via intraperitoneal injections. All studies were conducted according to internationally validated guidelines; some non-GLP published studies gave negative results, while two non-GLP studies were positive in mice treated intraperitoneally with dose levels in the range of the intraperitoneal LD_{50} for mice, one study presenting major flaws. No genotoxic effects on germ cells were detected in rats or mice treated orally at dose levels up to 2000 mg/kg bw. The induction of DNA strand breaks observed in mice treated intraperitoneally with doses close to or in excess of the LD_{50} has been associated to secondary effects of cytotoxicity (JMPR 2006; Kier 2015). Modes of action associated with secondary cytotoxicity should be excluded from the assessment of the intrinsic genotoxicity potential (Bryce et al. 2014; Kitamoto et al. 2015).

IARC combines information on glyphosate and glyphosate-based formulations, compiling studies on humans, other mammals, other vertebrates, invertebrates, and plants. Regarding *in vivo* mammalian studies, IARC reports positive effects for 5 out of 11 studies; four negative studies on micronucleus formation and dominant lethal mutation reported by JMPR (2006) are not included in the IARC evaluation. Positive effects are described only for intraperitoneal administrations at doses of 300 mg/kg bw. Although these effects had been previously postulated as secondary to (cyto)toxicity (Heydens et al. 2008; JMPR 2006), the role of (cyto)toxicity is not discussed in the IARC monograph. Positive effects are mostly observed in the liver, an organ that is considered inappropriate for assessing *in vivo* genotoxic effects after intraperitoneal administration (JMPR 2006).

A recent meta-analysis on micronuclei frequency (Ghisi et al. 2016) has confirmed that positive effects are limited to intraperitoneal administrations, and that the response is much higher for glyphosate-based formulations than for the active substance. Cytotoxicity of the surfactants added

to the formulations is presented as a plausible explanation, while the cytotoxicity of glyphosate in intraperitoneal administrations at high doses is not discussed. Significant differences are observed for males but not for females, a general difference is reported in the comparison of mammalian and non-mammalian systems, although similar responses are observed for mice and crocodilians (Ghisi et al. 2016).

Non-genotoxic modes of action

Non-genotoxic modes of action for carcinogenicity are assumed for about 9% of IARC classifications (Hernandez et al. 2009) and include endocrine disruption, tumour promotion, tissue-specific toxicity and inflammation, cytotoxicity and immune suppression, inhibition of gap-junction intercellular communications (GJICs), and other mechanisms (Benigni et al. 2013; Hernandez et al. 2009).

In the EU evaluation, the lack of evidence for carcinogenic potential of glyphosate meant that no further thought regarding the mode of action was considered necessary. IARC assessed the "key characteristics of human carcinogens" (Smith et al. 2016), concluding that there is weak evidence for receptor-mediated effects, cell proliferation or death, and immune effects, and strong evidence of oxidative stress.

Role of surfactants and other co-formulants

The EU assessment focuses on glyphosate, aiming to establish the properties of the active substance to be considered in the assessment of each formulation by individual Member States. IARC has a different approach, addressing both glyphosate and its formulations. The potential role of the co-formulants, which differ among formulations, is not assessed; however, the IARC monograph reports a large number of mechanistic studies with negative results for glyphosate but positive results for glyphosate-based formulations, as well as differences between formulations containing similar concentrations of glyphosate, indicating that other ingredients could lead to the effects observed when testing formulations (Coalova et al. 2014; Cox and Surgan 2006). Similar results are observed for other pesticides and particularly for herbicides (Cavas 2011); this is not surprising, as the mode of action leading the herbicidal activity is usually not linked to the toxicological profile in mammals.

Surfactants are frequently used in herbicide formulations, including glyphosate. Polyethoxylated tallowamines are several orders of magnitude more cytotoxic than glyphosate (Mesnage et al. 2013); the mode of action is cell death with inhibition of the mitochondrial succinate dehydrogenase activity and membrane damage leading to necrosis. This mode of action is different from glyphosate, while similar to that observed for glyphosate-based formulations (Benachour and Seralini 2009). These tallowamines also produce oxidative and DNA damage (Nobels et al. 2011), and increase the apoptotic potential of glyphosate (Kim et al. 2013). Other surfactants as well as solvents used in pesticides formulations are cytotoxic and, possibly, genotoxic (Nobels et al. 2011).

The cytotoxicity and potential genotoxicity of other ingredients should be considered before assuming that the effects observed for a formulated product are linked to the active substance. Secondary genotoxic effects produced by cytotoxicity should also be distinguished from true genotoxic potential (Bryce et al. 2014; Kitamoto et al. 2015). In fact, the UN and EU guidance recommends carcinogenicity and genotoxicity studies to be conducted on individual chemicals, limiting testing of mixtures/formulations to cases where synergistic effects are expected (United Nations 2015).

From hazard assessment to public health risk assessment

While IARC focuses exclusively on the hazard identification, regulatory assessments also include the estimation of the toxicological potency of the substance and the setting of toxicological reference values to be used in the human health risk assessment. The toxicological reference values offer quantitative indications of the toxicity of a chemical, indicating the levels of human exposure that, according to the current scientific knowledge, are considered acceptable from a regulatory perspective. The recent EFSA evaluation has changed significantly the toxicological profile of glyphosate, compared to the previous EU assessment (Table 8).

The Acute Reference Dose (ARfD) and Acceptable Daily Intake (ADI) represent oral doses that should not be exceeded in a single event (or repeated within 24 h) or daily in long term exposures, respectively. The Acceptable Operator Exposure Level (AOEL) represents a systemic daily dose that should not be exceeded in non-dietary exposures. Figure 1 visualises the current and previous EU toxicological reference values for glyphosate, compared with those established for the entire group of herbicides assessed in the EU. The ranking and percentile within the distribution of ca. 150 herbicides assessed in the EU (data extracted from the EU pesticides database http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN) gives an indication of the relative toxicity of glyphosate to humans compared to the other herbicides. In contrast with previous evaluations, effects produced after acute exposures were considered relevant, requiring an ARfD and an acute risk assessment (European Food Safety Authority 2015b). The human, animal and mechanistic

Table 8 Summary of the recent EU toxicological assessment of glyphosate and derivation of reference doses of risk assessment

	Relevant endpoints mg/kg body weight (per day)	Uncertainty factor	Reference dose for risk assessment mg/kg bw (per day)
Chronic dietary toxicity	Rat overall NOAEL: 100	100	Acceptable Daily Intake (ADI): 0.5
Acute dietary toxicity	Mice overall NOAEL: 150	100	Acute Reference Dose (ARfD): 0.5
Chronic non-dietary toxicity	Rodent reproductive NOAEL: 300 Rat neurotoxicity NOAEL: 617 Dog short-term NOAEL: 300 Critical endpoint: Rabbit NOAEL: 50 (maternal and developmental, also relevant for short-term exposures)	100 × 5 (accounting for 20% oral absorption)	Acceptable Operator Exposure Level (AOEL): 0.1

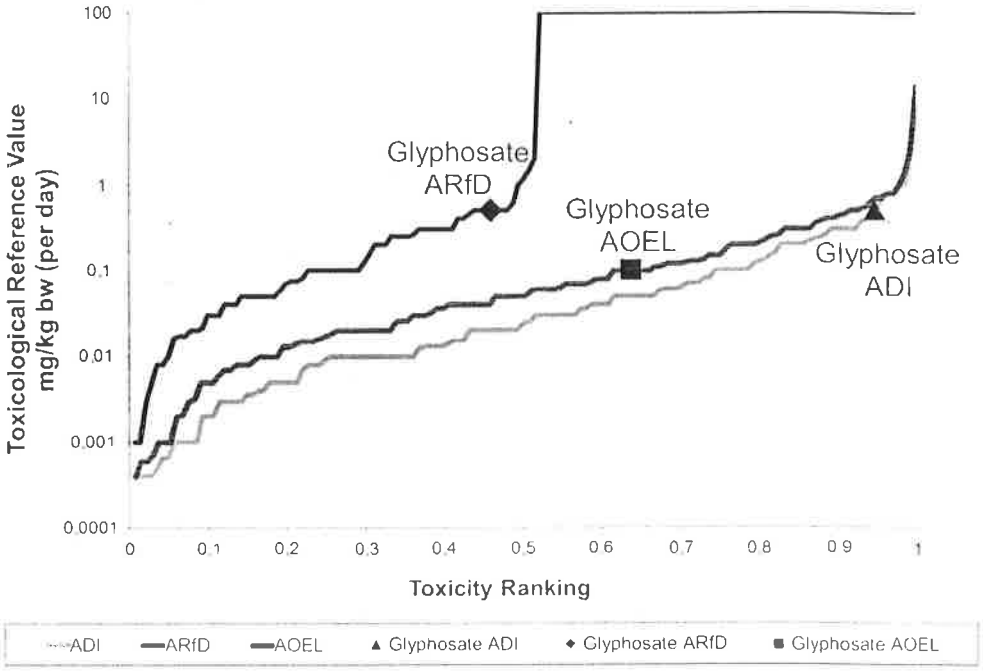

Fig. 1 Graphical representation of the EFSA proposed changes in the glyphosate toxicological profile expressed as the relative toxicity ranking. This ranking represents the percentile of each glyphosate's Toxicological Reference Value within the distribution of 141 herbicides assessed in the EU (data extracted from the EU pesticides database. http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=activesubstance.selection&language=EN on 25 May 2016)

evidence indicates that glyphosate cannot be considered as a potent DNA reactive tumour-initiating chemical, and that a risk assessment based on threshold toxicological reference values is scientifically valid (SCOEL 2013). The data summarised in Tables 4, 5 and 6 confirms that the proposed reference values (Table 8) provide sufficient protection for all effects observed in the carcinogenicity and long-term toxicity studies, including the trends for tumour induction considered as sufficient evidence by IARC.

Glyphosate has a relative low long-term dietary toxicity, being within the 10% of herbicides with higher ADI. Regarding short-term dietary exposure, the EU assessment proposed an ARfD which ranks glyphosate as slightly more toxic (45th percentile) than the average for herbicides. This new toxicological profile requires the re-assessment of health risks, which had only considered chronic exposure until now (Shao-Wen and Chun-Hong 2015). The need for personal protective equipment for glyphosate applicators is identified in the EFSA Conclusion. The need for an ARfD triggers also new considerations regarding the role of sporadic AOEL exceedance when addressing the risk of short-term inhalation and dermal exposures during application, including bystander and resident exposure in aerial applications, which are standard practice outside the EU in forest (Rolando et al. 2013) and for the control of illegal crops (Benner et al. 2016). Exposure estimations for children entering the area after application (Solomon et al. 2007) are higher than the proposed toxicological threshold.

Regarding residues in food, a comprehensive update of the dietary risk assessment will be performed in the EU, following the decision on the approval of glyphosate, covering all EU uses and the residues expected on imported food. Meanwhile, Niemann et al. (2015) have compiled information on human biomonitoring data, and concluded that current exposures are well below the toxicological references values; exposure of European

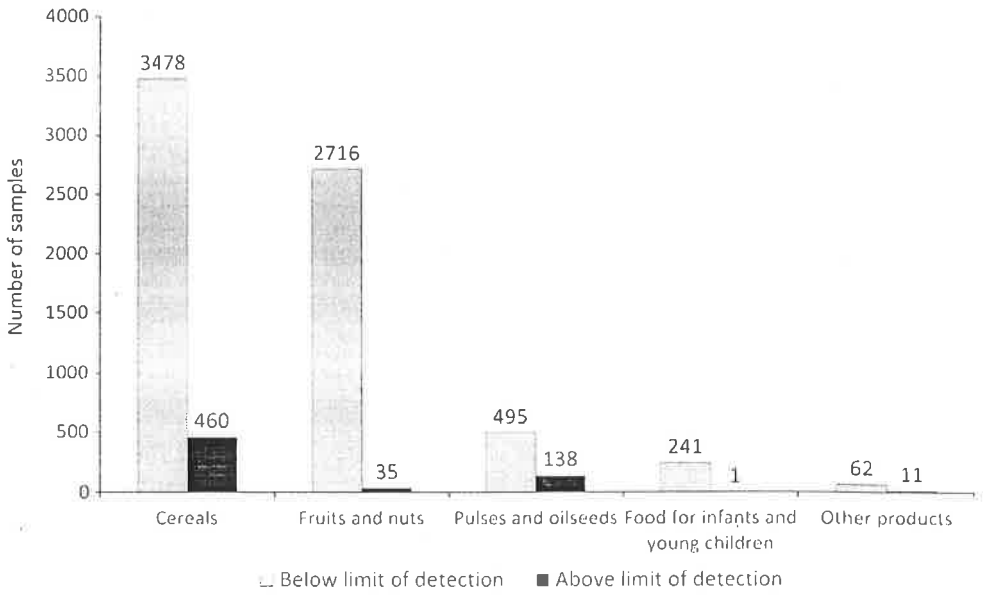

Fig. 2 Summary of EU monitoring data on glyphosate residues in food (2012–2014)

citizens seems to be lower than that of Americans. To complement these estimations, an indicative consumer exposure assessment based on EU monitoring data for glyphosate residues in food generated by competent authorities in the EU Member States is described below. The assessment covers over 10,000 samples of different types of food analysed for glyphosate residues between 2012 and 2014 (Fig. 2). Member States focussed the control activities for glyphosate mainly on crops relevant for human consumption, where the presence of glyphosate was expected, such as cereals (almost 4000 samples), followed by fruits, vegetables, pulses and oilseeds; it should be noted that only limited information is available on feed products such as soya beans (only nine soya beans samples were analysed). Overall glyphosate was detected in 6.3% of the samples, mostly in cereals (11.7% of the samples analysed contained residues above the Limit of Quantification), but also in lentils, linseed and table grapes, mostly from outside the EU. The legal limits were exceeded in 0.2% of the samples analysed for glyphosate. A very conservative risk assessment screening has been conducted with the EFSA PRIMO model (European Food Safety Authority 2007), using conservative assumptions. Table 9 summarises the residue levels measured in food items which were identified as main contributors in the risk assessment using European food consumption data. The data have been extracted from the EU pesticides residues monitoring programme (European Food Safety Authority 2016). Detailed information is provided in the supporting information.

Table 9 Glyphosate residue levels reported for the food items contributing with over 0.1% of the ADI or 2% of the ARfD in the European consumers' risk assessment (EFSA 2016)

Food item	Number of samples analysed for glyphosate	Percentage of samples with residues > LOQ	Maximum level mg/kg	Mean value mg/kg
Apples	215	1.9	0.10	0.02[$]
Barley	188	18.6	8.00	0.24
Beans (dry)	132	11.4%	4.00	0.16
Beans (with pods)	123	0.8%	0.05	0.02[$]
Lentils (dry)	277	30.3%	19.00	0.59
Oranges	192	0.5%	0.10	0.03[$]
Peas (dry)	41	37.7%	6.39	0.59
Peas (with pods)	38	7.9%	1.40	0.13
Peas (without pods)	22	0%	0.10	0.04[$]
Potatoes	88	0%	0.10	0.02[$]
Rye	557	4.1%	3.40	0.13
Wheat	2318	13.2%	4.00	0.14

[$]The mean value is similar to the Limit of Quantification

The acute risk assessment used the maximum reported result. The chronic risk assessment used mean residue concentrations, assuming that residues below the Limit of Quantification (LOQ) actually occurred in concentrations equivalent to the LOQ; considering that over 94% of the samples analysed did not contain residues above the LOQ, this assumption contributes to the conservatism of the estimated exposure. The chronic exposure was well below the ADI (0.5% for unprocessed products and 0.6% of the ADI when processed foods are included). In the acute risk assessment, the highest exposure was calculated for lentils (23.4% of the ARfD), followed by beans (14.6%) and wheat (11.6%). Pending on the on-going EFSA assessment, these estimations further support the conclusion that glyphosate residues in food do not represent a public health concern for European citizens.

Conclusions

The following main factors should be considered when explaining the differences between IARC and the EU evaluations: the evidence and information sources, the methodology and the overall aim. The comparison is summarised in Table 10.

Evidence in humans

The same epidemiological studies were used in both assessments; all studies focussed on farmers exposed to formulations. For pesticides, the regulatory dossier may include information on medical surveillance and epidemiological studies on manufacturing plant personnel directly exposed to the active substance; but this was not the case for glyphosate. The key IARC role in compiling and evaluating human evidence is well proven, and the EU assessment was updated to consider recent publications included in the IARC monograph. The same weak evidence in humans for the carcinogenicity of glyphosate was interpreted differently by IARC and EFSA. IARC considered the association between exposure to glyphosate and non-Hodgkin lymphoma as "limited evidence in humans"; while in the EU assessment, most experts considered the evidence as "very limited" and insufficient for triggering the classification. The difference in the interpretation between IARC and the EU is mainly related to the fact that IARC is because IARC considered that glyphosate is carcinogenic in animals, and concluded that strong evidence for two mechanisms, genotoxicity and oxidative stress, supported the plausibility of the weak association in humans.

Evidence on carcinogenicity in experimental animal models

Regarding animal carcinogenicity, three main aspects should be considered for understanding the different conclusions from IARC and EFSA. Lack of consistency among studies on the same species and strain at equivalent doses supported the conclusion of chance results in the EU evaluation. IARC, however, could not use some studies included in the EU evaluation, since the EU assessment was on-going and only a draft was available at the time of the IARC Working Group meeting, limiting the capacity for checking consistency among studies. Second, the lack of consistency between sexes; according to the UN-GHS criteria, a plausible sex-related mechanism should be investigated in these cases, and was not identified in the EU assessment. No specific guidance is provided in the IARC evaluation and no indication is provided in the monograph. Third, the role of secondary effects observed at doses with excessive toxicity. For regulatory assessments, when classification is linked to labelling and risk management options, secondary effects due to excessive doses are excluded as the assessment focuses on the intrinsic capacity of the chemical to induce tumours at lower, less toxic doses. This element is not described in the IARC methodology, and the IARC Working Group considered as positive trends those triggered by tumour incidences at doses with demonstrated excessive toxicity. Regulatory assessments have access to full study reports; for IARC, unpublished industry-sponsored studies are secondary information sources, and their use is limited to the study summaries from previous assessments published by other agencies. Despite not having access to the original study reports, the IARC Working Group was able to run new statistical analyses, although its capacity for verifying details relevant for assessing the biological relevance was limited by the level of detail provided in the reports published by the regulatory agencies. The comparison with the WHO expert group JMPR assessments for glyphosate, conducted in 2004 and 2016, is informative regarding the value of granting the experts access to the full study reports.

Evidence on genotoxicity and other mechanisms of carcinogenicity

Regarding sources of mechanistic information, genotoxicity/mutagenicity should be discussed independently of other possible mechanisms. As observed for glyphosate, both industry-sponsored and scientific publications offer relevant information on the genotoxicity potential of pesticides that has raised interest among the scientific community. On one hand, IARC included one industry-sponsored study reported by the US-EPA but not those reported by

Table 10 Comparative summary of IARC and EU assessments and conclusions

Issue	IARC	EU regulatory assessment
Epidemiological studies		
Evidence	Same human evidence based on published epidemiological studies. Different animal and mechanistic conclusions in the plausibility assessment	
Assessment	Positive and negative associations. Associations considered biologically plausible	Positive and negative associations. Associations considered week and lacking biological plausibility
Conclusion	Sufficient for "Limited evidence" in humans	Contradictory evidence, insufficient to be considered as "limited evidence"
Animal carcinogenicity		
Evidence (see Table 4)	US EPA and JMPR reports summarising industry studies results	Full industry study reports, covering a larger data set for mice and rats
Assessment (see Tables 5, 6)	Positive trends in one sex in some studies. Pair-wise comparisons without dose–response. No indication on consistency assessment between studies, sexes or consideration of excessive toxicity	Large data set with mostly negative findings. Positive findings were inconsistent (between sexes, statistical approaches, and among studies), observed only at very high doses above the Maximum Tolerable Dose, or lack of dose response
Conclusion	Sufficient evidence for carcinogenicity in animals	Unlikely to be carcinogenic in animals according to UN GHS weight of evidence
Genotoxicity		
Evidence	5 published *in vivo* studies on mammals, 1 secondary reference to industry studies and studies on formulations. Large coverage of non-mammalian species and formulations	Focus on 16 *in vivo* studies on mammals; guideline studies supported by additional published studies. Assessment limited to mammals and glyphosate active substance
Assessment	Biomarkers of DNA adducts and various types of chromosomal damage generally positive in the liver but only at high intraperitoneal doses (300 mg/kg bw) with mixed results for the kidney and bone marrow Inconsistent effects between glyphosate and glyphosate formulations reported for several studies, but not further assessed	Positive clastogenic effects in 2 out of 6 intraperitoneal studies at high toxic doses (above i.p. LD_{50}) in studies showing methodological deficiencies. 1 weak positive out of 8 oral studies limited to high dose, one sex, and high SD Positive results in indicative studies such as DNA strand breaks do not detect mutagenicity, rather cytotoxicity Consistent negative results for gene mutation in both bacteria and mammalian cells
Conclusion	Strong evidence that exposure to glyphosate is genotoxic	Unlikely to be genotoxic in humans. No classification for mutagenicity
Overall conclusion on carcinogenicity		
Hazard	Probably carcinogenic in humans. IARC Group 2A	Unlikely to be carcinogenic in humans. No classification as carcinogen

SD standard deviation

JMPR (JMPR 2006); on the other hand, IARC reviewed effects observed in non-mammalian systems, which were considered of limited relevance for the assessment of carcinogenicity in humans in the regulatory assessment. IARC also assessed glyphosate-based formulations.

An important difference among IARC and regulatory assessment is the identification of a non-threshold genotoxic mode of action for carcinogenicity. This is not part of the IARC evaluation, while for regulatory assessment this is a key element triggering the risk assessment methodology. The IARC monograph used genotoxicity and oxidative stress as supporting mechanistic evidence; according to IARC principles, no indication is provided regarding threshold or non-threshold modes of action. The IARC allocation in group 2A may suggest that for the IARC Working Group the evidence on genotoxicity was insufficient for considering glyphosate as a potent DNA reactive non-threshold genotoxic human carcinogen. In fact, all oral studies, even at very high doses, are negative and the only *in vivo* mammalian positive evidence was for intraperitoneal studies at very high doses at which (cyto)toxicity is expected. This is again linked to the consideration of secondary effects due to severe systemic toxicity described above for the animal studies, which should be excluded for the classification of genotoxicity and carcinogenicity according to the UN-GHS criteria.

Other mechanistic studies should be discussed in connection with the methodological approach. With the exception of genotoxicity, mechanistic data on the mode of action are used in the regulatory context for assessing the relevance for humans, and are mostly used to downgrade the classification (Boobis et al. 2006; Clewell 2005; Meek et al. 2014a). Mechanistic data can be pivotal in IARC evaluations with inconclusive evidence in humans (Cogliano et al. 2008; Lauby-Secretan et al. 2016); and IARC has used mechanistic data for upgrading 52 agents and downgrading 8 agents (Cogliano et al. 2008). The recent review of the IARC approach for assessing mechanistic information may further change this picture. Strong evidence on non-genotoxic mechanisms is included in the recent IARC assessments for lindane, DDT and 2,4-D (Loomis et al. 2015). Moreover, mechanistic information is essential in the assessment of causality *versus* chance and bias.

To summarise, definitions for limited and sufficient evidence in humans and animals are identical for IARC and the UN-GHS; however, differences in criteria and methodological considerations for weighing and assessing the evidence can lead to divergent interpretations between the IARC assessment and regulatory evaluations following the UN-GHS criteria, even when based on the same evidence.

The differences between IARC and regulatory assessments are related not only to parallel historical developments, but to the different overall scope. IARC classifications represent a first step, alerting on the carcinogenicity potential of a broad range of agents; scientific regulatory assessments are connected to specific risk management recommendations, such as labelling, packaging requirements, use restrictions, etc., and produce the basis to be used in the risk assessment. In this different context, the focus and role of conservativeness is very different. While IARC assessments are not connected to risk management decisions, and are based exclusively on published information, without access to the full study reports for regulated products, regulatory assessments may identify data gaps and request additional studies to confirm or exclude potential concerns identified during their evaluation.

Human health safety is a critical issue for understanding the consequences of scientific divergences regarding the carcinogenicity classification of glyphosate. Regulatory assessments cover all relevant effects, not only carcinogenicity. Effects other than tumour induction were responsible for setting the NOAELs of the long-term toxicity–carcinogenicity studies, and the toxicological reference values were established from critical effects observed at lower dose levels in other studies. From a health assessment perspective, the IARC-EFSA scientific divergence is at lower dose levels that are in reality of limited, if any, relevance. The toxicological reference values proposed by EFSA provide a margin of protection of about four orders of magnitude for the trends in tumour induction and genotoxic damage at toxic levels reported by IARC. Those effects are expected only in concomitance with other signs of toxicity and at exposure levels orders of magnitude higher than the toxicological reference values recommended by EFSA. Risk assessments based on human biomonitoring and monitoring of levels of glyphosate residues in food have not identified concerns for consumers, and a full consumers' risk assessment of all EU uses is on-going.

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574

Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA)

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The International Agency for Research on Cancer (IARC) Monographs Programme identifies chemicals, drugs, mixtures, occupational exposures, lifestyles and personal habits, and physical and biological agents that cause cancer in humans and has evaluated about 1000 agents since 1971. Monographs are written by ad hoc Working Groups (WGs) of international scientific experts over a period of about 12 months ending in an eight-day meeting. The WG evaluates all of the publicly available scientific information on each substance and, through a transparent and rigorous process,[1] decides on the degree to which the scientific evidence supports that substance's potential to cause or not cause cancer in humans.

For Monograph 112,[2] 17 expert scientists evaluated the carcinogenic hazard for four insecticides and the herbicide glyphosate.[3] The WG concluded that the data for glyphosate meet the criteria for classification as a *probable human carcinogen*.

The European Food Safety Authority (EFSA) is the primary agency of the European Union for risk assessments regarding food safety. In October 2015, EFSA reported[4] on their evaluation of the Renewal Assessment Report[5] (RAR) for glyphosate that was prepared by the Rapporteur Member State, the German Federal Institute for Risk Assessment (BfR). EFSA concluded that 'glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential'. Addendum 1 (the BfR Addendum) of the RAR[5] discusses the scientific rationale for differing from the IARC WG conclusion.

Serious flaws in the scientific evaluation in the RAR incorrectly characterise the potential for a carcinogenic hazard from exposure to glyphosate. Since the RAR is the basis for the European Food Safety Agency (EFSA) conclusion,[4] it is critical that these shortcomings are corrected.

THE HUMAN EVIDENCE

EFSA concluded 'that there is very limited evidence for an association between glyphosate-based formulations and non-Hodgkin lymphoma (NHL), overall inconclusive for a causal or clear associative relationship between glyphosate and cancer in human studies'. The BfR Addendum (p. ii) to the EFSA report explains that 'no consistent positive association was observed' and 'the most powerful study showed no effect'. The IARC WG concluded there is *limited evidence of carcinogenicity in humans* which means "A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence."[1]

The finding of *limited evidence* by the IARC WG was for NHL, based on high quality case–control studies, which are particularly valuable for determining the carcinogenicity of an agent because their design facilitates exposure assessment and reduces the potential for certain biases. The Agricultural Health Study[6] (AHS) was the only cohort study available providing information on the carcinogenicity

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of glyphosate. The study had a null finding for NHL (RR 1.1, 0.7–1.9) with no apparent exposure–response relationship in the results. Despite potential advantages of cohort versus case–control studies, the AHS had only 92 NHL cases in the unadjusted analysis as compared to 650 cases in a pooled case–control analysis from the USA.[7] In addition, the median follow-up time in the AHS was 6.7 years, which is unlikely to be long enough to account for cancer latency.[8]

The RAR classified all of the case–control studies as 'not reliable,' because, for example, information on glyphosate exposure, smoking status and/or previous diseases had not been assessed. In most cases, this is contrary to what is actually described in the publications. Well-designed case–control studies are recognised as strong evidence and routinely relied on for hazard evaluations.[9 10] The IARC WG carefully and thoroughly evaluated all available epidemiology data, considering the strengths and weaknesses of each study. This is key to determining that the positive associations seen in the case–control studies are a reliable indication of an association and not simply due to chance or methodological flaws. To provide a reasonable interpretation of the findings, an evaluation needs to properly weight studies according to quality rather than simply count the number of positives and negatives. The two meta-analyses cited in the IARC Monograph[11] are excellent examples of objective evaluations and show a consistent positive association between glyphosate and NHL.

The final conclusion[5] (Addendum 1, p.21) that "there was no unequivocal evidence for a clear and strong association of NHL with glyphosate" is misleading. IARC, like many other groups, uses three levels of evidence for human cancer data.[1] *Sufficient evidence* means 'that a causal relationship has been established' between glyphosate and NHL. BfR's conclusion is equivalent to deciding that there is not *sufficient evidence*. Legitimate public health concerns arise when 'causality is credible', that is, when there is *limited evidence of carcinogenicity*.

EVIDENCE FROM ANIMAL CARCINOGENICITY STUDIES

EFSA concluded 'No evidence of carcinogenicity was confirmed by the majority of the experts (with the exception of one minority view) in either rats or mice due to a lack of statistical significance in pairwise comparison tests, lack of consistency in multiple animal studies and slightly increased incidences only at dose levels at or above the limit dose/maximum tolerated dose (MTD), lack of preneoplastic lesions and/or being within historical control range'. The IARC WG review found a significant positive trend for renal tumours in male CD-1 mice,[12] a rare tumour, although no comparisons of any individual exposure group to the control group were statistically significant. The WG also identified a significant positive trend for hemangiosarcoma in male CD-1 mice,[13] again with no individual exposure group significantly different from controls. Finally, the WG also saw a significant increase in the incidence of pancreatic islet cell adenomas in two studies in male Sprague-Dawley rats.[14–16] In one of these rat studies, thyroid gland adenomas in females and liver adenomas in males were also increased. By the IARC review criteria,[1] this constitutes *sufficient evidence* in animals.

The IARC WG reached this conclusion using data that were publicly available in sufficient detail for independent scientific evaluation (a requirement of the IARC Preamble[1]). On the basis of the BfR Addendum, it seems there were three additional mouse studies and two additional rat studies that were unpublished and available to EFSA. Two of the additional studies were reported to have a significant trend for renal tumours, one in CD-1 mice (Sugimoto. *18-Month Oral Oncogenicity Study in Mice*. Unpublished, designated ASB2012–11493 in RAR. 1997), and one in Swiss-Webster mice (Unknown. *A chronic feeding study of glyphosate (roundup technical) in mice*. Unpublished, designated ABS2012–11491 in RAR. 2001). One of these studies (Sugimoto. Unpublished, 1997) also reported a significant trend for hemangiosarcoma. The RAR also reported two studies in CD-1 mice showing significant trends for malignant lymphoma (Sugimoto. Unpublished, 1997; Unknown. *Glyphosate Technical: Dietary Carcinogencity Study in the Mouse*. Unpublished, designated ABS2012–11492 in RAR. 2009).

The RAR dismissed the observed trends in tumour incidence because there are no individual treatment groups that are significantly different from controls and because the maximum observed response is reportedly within the range of the historical control data (Table 5.3–1, p.90). Care must be taken in using historical control data to evaluate animal carcinogenicity data. In virtually all guidelines,[1 17 18] scientific reports[19] and publications[20–23] on this issue, the recommended first choice is the use of concurrent controls and trend tests, even in the EC regulations cited in the RAR[18] (see p.375). Trend tests are more powerful than pairwise comparisons, particularly for rare tumours where data are sparse. Historical control data should be from studies in the same time frame, for the same animal strain, preferably from the same laboratory or the same supplier and preferably reviewed by the same pathologist.[17 18] While the EFSA final peer review[4] mentions the use of historical control data from the original laboratory, no specifics are provided and the only referenced historical control data[24] are in the BfR addendum.[5] One of the mouse studies[12] was clearly done before this historical control database was developed, one study (Sugimoto. Unpublished, 1997) used Crj:CD-1 mice rather than Crl:CD-1 mice, and one study[13] did not specify the substrain and was reported in 1993 (probably started prior to 1988). Hence, only a single study (Unknown. Unpublished, 2009) used the same mouse strain as the cited historical controls, but was reported more than 10 years after the historical control data set was developed.

The RAR dismissed the slightly increased tumour incidences in the studies considered because they occurred "only at dose levels at or above the limit dose/maximum tolerated dose (MTD)", and because there was a lack of preneoplastic lesions. Exceeding the MTD is demonstrated by an increase in mortality or other serious toxicological findings at the highest dose, not by a slight reduction in body weight. No serious toxicological findings were reported at the highest doses for the mouse studies in the RAR. While some would argue that these high doses could cause cellular disruption (eg, regenerative hyperplasia) leading to cancer, no evidence of this was reported in any study. Finally, a lack of preneoplastic lesions for a significant neoplastic finding is insufficient reason to discard the finding.

MECHANISTIC INFORMATION

The BfR Addendum dismisses the IARC WG finding that 'there is strong evidence that glyphosate causes genotoxicity' by suggesting that unpublished evidence not seen by the IARC WG was overwhelmingly negative and that, since the reviewed studies were not done under guideline principles, they should get less weight. To maintain transparency, IARC reviews only publicly available data. The use of confidential data submitted to the BfR makes it impossible for any scientist not associated with BfR to review this conclusion. Further weakening their interpretation,

the BfR did not include evidence of chromosomal damage from exposed humans or human cells that were highlighted in Tables 4.1 and 4.2 of the IARC Monograph.[3]

The BfR confirms (p.79) that the studies evaluated by the IARC WG on oxidative stress were predominantly positive but does not agree that this is strong support for an oxidative stress mechanism. They minimise the significance of these findings predominantly because of a lack of positive controls in some studies and because many of the studies used glyphosate formulations and not pure glyphosate. In contrast, the WG concluded that (p.77) 'Strong evidence exists that glyphosate, AMPA and glyphosate-based formulations can induce oxidative stress'. From a scientific perspective, these types of mechanistic studies play a key role in distinguishing between the effects of mixtures, pure substances and metabolites.

Finally, we strongly disagree that data from studies published in the peer-reviewed literature should automatically receive less weight than guideline studies. Compliance with guidelines and Good Laboratory Practice does not guarantee validity and relevance of the study design, statistical rigour and attention to sources of bias.[25 26] The majority of research after the initial marketing approval, including epidemiology studies, will be conducted in research laboratories using various models to address specific issues related to toxicity, often with no testing guidelines available. Peer-reviewed and published findings have great value in understanding mechanisms of carcinogenicity and should be given appropriate weight in an evaluation based on study quality, not just on compliance with guideline rules.

GENERAL COMMENTS

Science moves forward on careful evaluations of data and a rigorous review of findings, interpretations and conclusions. An important aspect of this process is transparency and the ability to question or debate the findings of others. This ensures the validity of the results and provides a strong basis for decisions. Many of the elements of transparency do not exist for the RAR.' For example, citations for almost all references, even those from the open scientific literature, have been redacted. The ability to objectively evaluate the findings of a scientific report requires a complete list of cited supporting evidence. As another example, there are no authors or contributors listed for either document, a requirement for publication in virtually all scientific journals where financial support, conflicts of interest and affiliations of authors are fully disclosed. This is in direct contrast to the IARC WG evaluation listing all authors, all publications and public disclosure of pertinent conflicts of interest prior to the WG meeting.[27]

Several guidelines have been devised for conducting careful evaluation and analysis of carcinogenicity data, most after consultation with scientists from around the world. Two of the most widely used guidelines in Europe are the OECD guidance on the conduct and design of chronic toxicity and carcinogenicity studies[17] and the European Chemicals Agency Guidance on Commission Regulation (EU) No 286/2011;[18] both are cited in the RAR. The methods used for historical controls and trend analysis are inconsistent with these guidelines.

Owing to the potential public health impact of glyphosate, which is an extensively used pesticide, it is essential that all scientific evidence relating to its possible carcinogenicity is publicly accessible and reviewed transparently in accordance with established scientific criteria.

SUMMARY

The IARC WG concluded that glyphosate is a 'probable human carcinogen', putting it into IARC category 2A due to *sufficient evidence* of carcinogenicity in animals, *limited evidence* of carcinogenicity in humans and *strong* evidence for two carcinogenic mechanisms.

- The IARC WG found an association between NHL and glyphosate based on the available human evidence.
- The IARC WG found significant carcinogenic effects in laboratory animals for rare kidney tumours and hemangiosarcoma in two mouse studies and benign tumours in two rat studies.
- The IARC WG concluded that there was strong evidence of genotoxicity and oxidative stress for glyphosate, entirely from publicly available research, including findings of DNA damage in the peripheral blood of exposed humans.

The RAR concluded' (Vol. 1, p.160) that 'classification and labelling for carcinogenesis is not warranted' and 'glyphosate is devoid of genotoxic potential'.

- EFSA[4] classified the human evidence as 'very limited' and then dismissed any association of glyphosate with cancer without clear explanation or justification.
- Ignoring established guidelines cited in their report, EFSA dismissed evidence of renal tumours in three mouse studies, hemangiosarcoma in two mouse studies and malignant lymphoma in two mouse studies. Thus, EFSA incorrectly discarded all findings of glyphosate-induced cancer in animals as chance occurrences.
- EFSA ignored important laboratory and human mechanistic evidence of genotoxicity.
- EFSA confirmed that glyphosate induces oxidative stress but then, having dismissed all other findings of possible carcinogenicity, dismissed this finding on the grounds that oxidative stress alone is not sufficient for carcinogen labelling.

The most appropriate and scientifically based evaluation of the cancers reported in humans and laboratory animals as well as supportive mechanistic data is that glyphosate is a *probable human carcinogen*. On the basis of this conclusion and in the absence of evidence to the contrary, it is reasonable to conclude that glyphosate formulations should also be considered likely human carcinogens.' The CLP Criteria[18] (Table 3.6.1, p.371) allow for a similar classification of Category 1B when there are 'studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals'.

In the RAR, almost no weight is given to studies from the published literature and there is an over-reliance on non-publicly available industry-provided studies using a limited set of assays that define the minimum data necessary for the marketing of a pesticide. The IARC WG evaluation of *probably carcinogenic to humans* accurately reflects the results of published scientific literature on glyphosate and, on the face of it, unpublished studies to which EFSA refers.

Most of the authors of this commentary previously expressed their concerns to EFSA and others regarding their review of glyphosate[28] to which EFSA has published a reply.[29] This commentary responds to the EFSA reply.

The views expressed in this editorial are the opinion of the authors and do not imply an endorsement or support for these opinions by any organisations to which they are affiliated.

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Competing interests CJP, MTS and DDW are providing advice to a US law firm involved in glyphosate litigation. CJP also works part-time for the Environmental Defense Fund on issues not related to pesticides.

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JECH

Differences in the carcinogenic evaluation of glyphosate between the International Agency for Research on Cancer (IARC) and the European Food Safety Authority (EFSA)

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Notes

Health and environmental impacts of glyphosate: July 2001

Monsanto* s claims	Independent research findings
Roundup has a low irritation potential for eye and skin and otherwise is not a risk to human health.	• Roundup is amongst the top most-reported pesticides causing poisoning incidents in several countries. • Roundup causes a range of acute symptoms including recurrent eczema, respiratory problems, elevated blood pressure and allergic reactions
Roundup does not cause any adverse reproductive effects	In laboratory tests on rabbits glyphosate caused long-lasting, harmful effects on semen quality and sperm counts.
Roundup is not mutagenic in mammals.	DNA damage has been observed in laboratory experiments in mice organs and tissue.
Roundup is environmentally safe	• In the agricultural environment, glyphosate is toxic to beneficial soil organisms and beneficial arthropod predators, and increases crops* susceptibility to diseases. • The use of glyphosate in forestry and agriculture has indirect harmful effects on birds and small mammals by damaging their food supplies and habitat. • Roundup containing POEA is lethal to the tadpoles of three species of tree and ground frogs in Australia. The Australian government has banned the use of these products near water. • Sub-lethal doses of glyphosate from spray-drift damages wildflower communities and can affect some species up to 20 metres away from the sprayer. • The use of glyphosate in arable areas causes dieback in hedgerow trees. • Glyphosate promotes population growth of a water snail that is the intermediate host of liver fluke in mammals. • The breakdown of glyphosate by micro-organisms in water may

	stimulate eutrophication effects.
Roundup is rapidly inactivated in the soil and water.	• Glyphosate is very persistent in soils and sediments. • Glyphosate inhibited the formation of nitrogen-fixing nodules on clover for 120 days after treatment. • Glyphosate residues were found in lettuce, carrot and barley when they were planted a year after glyphosate was applied. • Phosphate fertilisers may inhibit breakdown in soil.
Roundup is immobile and does not leach from soils.	• Glyphosate can readily desorb from soil particles in a range of soil types. It can be extensively mobile and leach to lower soil layers. • Glyphosate can be carried by soil particles suspended in run off.
Roundup does not contaminate drinking water when used by local authorities on hard surfaces.	In the UK, levels of glyphosate above the EU limit have been detected by the Welsh Water Company every year since 1993. The Drinking Water Inspectorate recommends that glyphosate be monitored, particularly in areas where it is used by local authorities on hard surfaces
It is nearly impossible for glyphosate resistance to evolve in weeds.	In 1996, glyphosate-resistant ryegrass was discovered in Australia.
Out crossing from GM crops and the transfer of novel genes occurs over a short distance and can be easily managed.	In those crops which have been examined, the densities of pollen are much higher and their dispersal patterns differ from large fields compared to those found in experimental plots. Wind dispersal of pollen occurs over much greater distances and at higher concentrations than predicted by experimental plots. Gene flow from transgenic oil seed crops is inevitable.
Roundup Ready crops will reduce levels of herbicide use.	Herbicide-tolerant crops will intensify and increase dependency on herbicide use in agriculture rather than lead to any significant reductions. A variety of herbicides will have to be reintroduced to control glyphosate-tolerant volunteers, feral populations of crops and resistant weed

583

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FOOD FOR THOUGHT Pesticides

Glyphosate Fact Sheet: Cancer and Other Health Concerns

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Posted on January 15, 2019 by Stacy Malkan

Glyphosate, a synthetic herbicide patented in 1974 by the Monsanto Company and now manufactured and sold by many companies in hundreds of products, has been associated with cancer and other health concerns. Glyphosate is best known as the active ingredient in Roundup-branded herbicides, and the herbicide used with "Roundup Ready" genetically modified organisms (GMOs).

Herbicide tolerance is the most prevalent GMO trait engineered into food crops, with some 90% of corn and 94% of soybeans in the U.S. engineered to tolerate herbicides, according to USDA data. A 2017 study found that Americans' exposure to glyphosate increased approximately 500 percent since Roundup Ready GMO crops were introduced in the U.S in 1996. Here are some key facts about glyphosate:

Most Widely Used Pesticide

According to a February 2016 study, glyphosate is the most widely used pesticide: "In the U.S., no pesticide has come remotely close to such intensive and widespread use." Findings include:

- Americans have applied 1.8 million tons of glyphosate since its introduction in 1974.
- Worldwide 9.4 million tons of the chemical has been sprayed on fields – enough to spray nearly half a pound of Roundup on every cultivated acre of land in the world.
- Globally, glyphosate use has risen almost 15-fold since Roundup Ready GMO crops were introduced.

Cancer Concerns

The scientific literature and regulatory conclusions regarding glyphosate and glyphosate-based herbicides show a mix of findings, making the safety of the herbicide a hotly debated subject:

In 2015, the **World Health Organization's International Agency for Research on Cancer (IARC)** classified glyphosate as "probably carcinogenic to humans" after reviewing years of published and peer-reviewed scientific studies. The team of international scientists found there was a particular association between glyphosate and non-Hodgkin lymphoma.

At the time of the IARC classification, the **Environmental Protection Agency (EPA)** was conducting a 1178 registration review. The EPA's Cancer Assessment Review Committee (CARC) issued a report in September 2016 concluding that glyphosate was "not likely to be carcinogenic to humans" at doses relevant to human health. In publishing the CARC report, the EPA said that it was beginning work with the National Toxicology Program to investigate the mechanisms and toxic effects of glyphosate formulations. The agency then convened a Scientific Advisory Panel (SAP) in December 2016 to review the CARC report conclusion that glyphosate was not likely to be carcinogenic. The scientific advisory panel members were divided in their assessment of EPA's work, with some finding the EPA erred in how it evaluated certain research. Additionally, the EPA's Office of Research and Development determined that the agency's Office of Pesticide Programs had not followed proper protocols in its evaluation of glyphosate. An ORD memo stated that the government scientists agreed in part with IARC and believed EPA was not properly following guidelines in coming to the conclusion that glyphosate was not likely to be carcinogenic. ORD said the evidence could be deemed to support a "likely" carcinogenic or "suggestive" evidence of carcinogenicity classification. Nevertheless the EPA issued a draft report on glyphosate in December 2017 continuing to hold that the chemical is not likely to be carcinogenic.

EFSA, ECA, WHO/FAO JMPR: The European Food Safety Authority and the European Chemicals Agency have said glyphosate is not likely to be carcinogenic to humans. But a March 2017 report by environmental and consumer groups argued that regulators relied improperly on research that was directed and manipulated by the chemical industry.

The WHO/FAO Joint Meeting on Pesticide Residues determined that glyphosate was unlikely to pose a carcinogenic risk to humans from exposure through the diet, though the finding was tarnished by conflict of interest concerns after it was revealed that certain members of the group, including its chair, worked for the International Life Sciences Institute, a group funded in part by Monsanto and one of its lobbying organizations.

California OEHHA: On March 28, 2017, the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment confirmed that it would add glyphosate to California's Proposition 65 list of chemicals known to cause cancer. Monsanto sued to block the action but the case was dismissed. In a separate case, the court found that California could not require cancer warnings for products containing glyphosate. On June 12, 2018, the United States District Court for the Eastern District of California denied the California Attorney General's request for the court to reconsider the decision. The court found that California could only require commercial speech that disclosed "purely factual and uncontroversial information," and the science surrounding glyphosate carcinogenicity was not proven.

Agricultural Health Study: A long-running U.S. government-backed prospective cohort study of farm families in Iowa and North Carolina has not found any connections between glyphosate use and non-Hodgkin lymphoma, but the researchers reported that "among applicators in the highest exposure quartile, there was an increased risk of acute myeloid leukemia (AML) compared with never users..." The most recent published update to the study was made public in late 2017.

New studies in 2019 report cancer links and concerns about the validity of the EPA classification:

- April 2019: the U.S. Agency for Toxic Substances and Disease Registry issued its draft toxicological profile for glyphosate. The report confirms increased cancer risk from glyphosate exposures. (Emails released via court proceedings show officials at EPA and Monsanto trying to stop the ATSDR report; read the emails here.)
- March 2019 study published in the International Journal of Epidemiology analyzed data from more than 30,000 farmers and agricultural workers from studies done in France, Norway and the U.S., and reported links between glyphosate and diffuse large B-cell lymphoma.
- February 2019: A new meta-analysis published in Mutation Research/Reviews in Mutation Research reported a "compelling link" between glyphosate-based herbicides and non-Hodgkin lymphoma. Three of the study authors were members of the EPA's scientific advisory panel on

glyphosate who have stated publicly that the EPA failed to follow proper scientific practices in its 1179 glyphosate assessment.

- January 2019: An analysis published in Environmental Sciences Europe argues that the U.S. EPA's classification of glyphosate disregarded substantial scientific evidence of genotoxicity (the negative impact on a cell's genetic material) associated with weed killing products such as Roundup.

Cancer Lawsuits

More than 11,000 people have filed suit against Monsanto Company (now Bayer) alleging that exposure to Roundup herbicide caused them or their loved ones to develop non-Hodgkin lymphoma, and that Monsanto covered up the risks. As part of the discovery process, Monsanto has had to turn over millions of pages of its internal records. We are posting these Monsanto Papers as they become available here. For news and tips about the ongoing legislation, see Carey Gillam's Roundup Trial Tracker. The first two trials ended with juries ruling that Monsanto's weed killer was a substantial contributing factor in causing the plaintiffs to develop non-Hodgkin lymphoma, and giving large awards for liability and damages.

Monsanto influence in research: In March 2017, the federal court judge unsealed some internal Monsanto documents that raised new questions about Monsanto's influence on the EPA process and about the research regulators rely on. The documents suggest that Monsanto's long-standing claims about the safety of glyphosate and Roundup do not necessarily rely on sound science as the company asserts, but on efforts to manipulate the science.

A study for the European Parliament published January 2019 asserts that the EU approval of glyphosate was based on plagiarized text from Monsanto. The study found plagiarism in 50.1 percent of chapters dealing with the assessment of published studies on health risks related to glyphosate in Germany's Federal Institute for Risk Assessment, including whole paragraphs and entire pages of plagiarized text.

More information about scientific interference:

- "The Monsanto Papers: Poisoning the Scientific Well," by Leemon McHenry (2018)
- "Roundup litigation discovery documents: implications for public health and journal ethics," by Sheldon Krimsky and Carey Gillam (June 2018)
- Letter to Nature by Stéphane Horel and Stéphane Foucart (March 2018)

Endocrine Disruption and Other Health Concerns

Some research suggests that glyphosate may be an endocrine disruptor. It has also been linked to liver disease, birth defects and reproductive problems in laboratory animals; and may kill beneficial gut bacteria and damage the DNA in human embryonic, placental and umbilical cord cells. A 2019 study in a Nature journal reported increases in obesity, reproductive and kidney diseases, and other problems in the second- and third-generation offspring of rats exposed to glyphosate. See the study and Washington State University press release.

Recent studies have shown adverse biological effects from low-dose exposures to glyphosate at levels to which people are routinely exposed.

- A birth cohort study in Indiana published in 2017 – the first study of glyphosate exposure in US pregnant women using urine specimens as a direct measure of exposure – found detectable levels of glyphosate in more than 90% of the pregnant women tested and found the levels were significantly correlated with shortened pregnancy lengths.
- A 2018 ecological and population study conducted in Argentina found high concentrations of glyphosate in the soil and dust in agricultural areas that also reported higher rates of spontaneous abortion and congenital abnormalities in children, suggesting a link between environmental

exposure to glyphosate and reproductive problems. No other relevant sources of pollution were identified. 1180

- A 2017 study associated chronic, very low-level glyphosate exposures to non-alcoholic fatty liver disease in rats. According to the researchers, the results "imply that chronic consumption of extremely low levels of a GBH formulation (Roundup), at admissible glyphosate-equivalent concentrations, are associated with marked alterations of the liver proteome and metabolome," the biomarkers for NAFLD.
- A 2018 rat study conducted by the Ramazzini Institute reported that low-dose exposures to Roundup at levels considered safe significantly altered the gut microbiota in some of the rat pups. Another 2018 study reported that higher levels of glyphosate administered to mice disrupted the gut microbiota and caused anxiety and depression-like behaviors.
- A 2018 rat study by Argentinian researchers linked low-level perinatal glyphosate exposures to impaired female reproductive performance and congenital anomalies in the next generation of offspring.

Glyphosate has also been linked by recent studies to harmful impacts on bees and monarch butterflies.

- A 2018 study reported that glyphosate damaged the beneficial gut bacteria in honeybees and made them more prone to deadly infections. This followed research from China showing that honeybee larvae grew more slowly and died more often when exposed to glyphosate, and a 2015 study that found field-levels of exposure impaired the cognitive capacities of honeybees.
- Research from 2017 correlated glyphosate use with reduced populations of monarch butterflies, possibly due to reductions in milkweed, the main food source for monarch butterflies.

Desiccation

Some farmers use glyphosate on non-GMO crops such as wheat, barley, oats, and lentils to dry down the crop ahead of harvest in order to accelerate the harvest. This practice, known as desiccation, may be a significant source of dietary exposure to glyphosate.

Glyphosate in Food: U.S. Drags Its Feet on Testing

The USDA quietly dropped a plan to start testing food for residues of glyphosate in 2017. Internal agency documents obtained by U.S. Right to Know show the agency had planned to start testing over 300 samples of corn syrup for glyphosate in April 2017. But the agency killed the project before it started. The U.S. Food and Drug Administration began a limited testing program in 2016, but the effort was fraught with controversy and internal difficulties and the program was suspended in September 2016. Both agencies have programs that annually test foods for pesticide residues but both have routinely skipped testing for glyphosate.

Before the suspension, one FDA chemist found alarming levels of glyphosate in many samples of U.S. honey, levels that were technically illegal because there have been no allowable levels established for honey by the EPA. Here is a recap of news about glyphosate found in food:

- October 2018: FDA issued its first-ever report showing the results of its glyphosate residue in food testing. The FDA said no residues of glyphosate were found in milk or eggs, but residues were found in 63.1 percent of corn samples and 67 percent of soybean samples, according to FDA data. The agency did not disclose in that report the findings of glyphosate in oatmeal or honey products.
- April 2018: internal FDA emails indicated the agency had trouble finding food sample without traces of glyphosate.

- Sept. 2016: FDA found glyphosate in US honey at double the levels allowed in the EU, and FDA tests confirm oatmeal and baby foods contain glyphosate.
- Nov. 2016: FDA chemist found glyphosate in honey in Iowa at 10X higher levels than allowed in EU. Also in November, independent testing by consumer group Food Democracy Now found glyphosate in Cheerios, oatmeal cookies, Ritz crackers and other popular brands at high levels.

Pesticides in Our Food: Where's the Safety Data?

USDA data from 2016 shows detectable pesticide levels in 85% of more than 10,000 foods sampled, everything from mushrooms to grapes to green beans. The government says there are little to no health risks, but some scientists say there is little to no data to back up that claim. See "Chemicals on our food: When "safe" may not really be safe: Scientific scrutiny of pesticide residue in food grows; regulatory protections questioned," by Carey Gillam (11/2018).

Statements From Scientists and Health Care Providers

- July 31, 2019 statement by the International Federation of Gynecology and Obstetrics (FIGO) Reproductive and Environmental Health Committee: "We recommend that glyphosate exposure to populations should end with a full global phase out."
- June 2017 essay by 14 scientists: "current safety standards for glyphosate-based herbicides are are outdated and may fail to protect public health and the environment" – Journal of Epidemiology and Community Health
- February 2016 consensus statement from scientists on concerns and risks associated with exposure to glyphosate-based herbicides – Environmental Health Journal

Food For Thought, Pesticides Bayer, cancer, endocrine disruption, EPA, European Chemicals Agency, European Food Safety Authority, glyphosate, GMO, IARC, ILSI, International Life Sciences Institute, JMPR, Joint Meeting on Pesticide Residues, Monsanto, non-Hodgkin lymphoma, Office of Pesticide Programs, Office of Research and Development, ORD, RoundUp, U.S. Food, U.S. Food and Drug Adminstration, USDA, USFDA

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588

U.S. RIGHT TO KNOW

PURSUING TRUTH AND TRANSPARENCY IN AMERICA'S FOOD SYSTEM

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REVIEW

Glyphosate toxicity for animals

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Abstract
Pesticides and herbicides gained popularity due to a strong need to curb the starvation of billions of humans. Glyphosate is the most commonly used herbicide and was considered to be non-toxic. But its use in excess in agricultural lands has polluted soils and waters. Nowadays, glyphosate residues are found in soil, water and food. As a result glyphosate causes severe acute and chronic toxicological effects. We review toxicological effects of glyphosate and metabolites on organisms of the kingdom animalia, both unicellular and multicellular organisms. Adverse effects on unicellular organisms have been established in many experiments. For instance, glyphosate has reduced the rate of photosynthesis in Euglena, has decreased the radial growth of mycorrhizal fungal species and is also reducing the profusion of certain bacteria present in rhizospheric microbial communities. Glyphosate poses serious threat to multicellular organisms as well. Its toxicological effects have been traced from lower invertebrates to higher vertebrates. Effects have been observed in annelids (earthworms), arthropods (crustaceans and insects), mollusks, echinoderms, fish, reptiles, amphibians and birds. Toxicological effects like genotoxicity, cytotoxicity, nuclear aberration, hormonal disruption, chromosomal aberrations and DNA damage have also been observed in higher vertebrates like humans.

Keywords Glyphosate · Toxicity · Excessive use · Herbicide · Environmental contamination

Introduction

Agrochemicals have become global necessity to increase crop productivity in agricultural fields. Nowadays, they play a pivotal role in controlling not only the pests and rodents but also many microbial infections. There are several types of herbicides, insecticides and pesticides that are in use in the modern cultivation of lands. Sadly, the surge in human needs and the greed for enhanced production of food yields has resulted in excessive consumption of these agrochemicals. Astonishingly, the initial use of pesticides began along with the "agricultural evolution" of mankind. According to definition of US Environmental Protection Agency, pesticide is any substance proposed for repelling, destroying, preventing, regulating or controlling pests (Taylor et al. 2007). Originally, natural and organic pesticides were used for pest control. However, after World War II, there was starvation all around and in order to boost the fight against hunger and malnutrition there was an urgent need to augment the crop productivity. This excessive demand from the contemporary agricultural infrastructure motivated the scientific fraternity to invent many synthetic chemicals which could shoot up the crop productions manifolds. The need of hour and the accomplishment of modern pesticide industry persuaded the widespread recognition of these synthetic chemicals around the world, and it led to subsequent dependence on them (Fishel and Ferrell 2013). Soon after its discovery in 1970, glyphosate (N-(phosphonomethyl) glycine) was initially accepted as an herbicide in 1974. Since then, it has globally become the most prominent herbicide. Looking into history, it was synthesized by Henri Martin of Swiss Pharmaceutical Company (Cilag). But its herbicidal properties were analyzed later on by John. E. Franz of Monsanto Company (Gill et al. 2017). Glyphosate is a broad spectrum, post-emergent,

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systemic and non-selective herbicide. It is used to kill several annual and perennial plants (Tu et al. 2001). Glyphosate-based herbicides are used to kill unwanted weeds from farmlands, but along with them, they also quell all the plants which are not genetically resistant to them. Glyphosate alone is not used as an herbicide; in fact it is always blended with different surfactants to increase its perforation into plant cells which adds toxicity to it (Monsanto International and Monsanto Europe 2010). It is used to repress superfluous plants (mostly weeds) and clear the space for the growth of vegetation in fields apart from enhancing the plantation in the parks, forests, railway lines, public streets and gardens. In spite of its tremendous benefits in controlling the weeds and having a reputation of being least toxic pesticide (Franz et al. 1997), its overuse has severely affected other nontarget organisms present in soil biota (Friends of Earth Europe 2013).

From various surveys conducted to determine the quantum of usage of glyphosate, it has been found that by 2014, annual consumption of glyphosate has increased to 240 million pounds. The annual consumption of glyphosate in last two decades has increased substantially (Fig. 1), and it is the most commonly used herbicide in USA (Myers et al. 2016). In other countries like Germany and Denmark, 35–39% of the agriculture depends on glyphosate (Steinmann et al. 2012). In Argentina, as well, glyphosate is the most frequently used herbicide, with annual usage of 180–200 million liters (Nedelkoska and Low 2004). However, with the evolution of glyphosate- resistant crops, the farmers all around the world have been forced to increase the use of this herbicide manyfold (CCM Information International Report 2011; Sansom 2012).

Earlier, it was contemplated to be non-carcinogenic in nature (Duke and Powles 2008). Ironically, a latest report by World Health Organisation (WHO) and Food and Agriculture Organisation (FAO) proved that glyphosate is responsible for non-Hodgkin's lymphoma in some case-control studies. However, large sample sizes have not yet shown any positive verification for this statement. The report also manifests that glyphosate is non-carcinogenic at lower doses, but it could not negate the possibility of cancer in rats at higher doses (Gill et al. 2017). Toxicity mechanisms of glyphosate are quite complicated and vary with its various formulations. Empirically, glyphosate has also shown very low oral and dermal toxicity, whereas, its toxicity by intraperitoneal route is very much evident (Bradberry et al. 2004).

Mode of action

Glyphosate kills the plant by hampering the biosynthesis of essential aromatic amino acids required for its growth. It hinders the production of enzyme

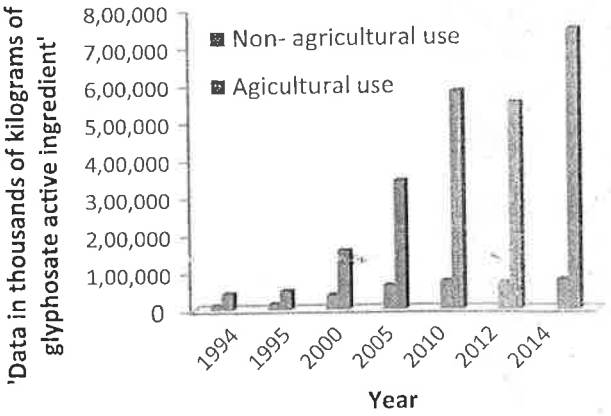

Fig. 1 The annual consumption of glyphosate throughout the world in the last two decade (from 1994 to 2014). Values shown are for global agricultural and non-agricultural use of glyphosate. Data from Benbrooke (2016)

5-enolpyruvylshikimate-3-phosphate synthase of shikimate pathway. Shikimate pathway is a metabolic pathway present in plants for the biosynthesis of aromatic amino acids (Gill et al. 2017). 5-Enolpyruvylshikimate-3-phosphate synthase is liable for the biogenesis of chorismate. Chorismate is the intermediate in the synthesis of aromatic amino acids (tryptophan, phenylalanine and tyrosine) (Williams et al. 2000). Glyphosate acts as antagonistic analog of phosphoenolpyruvate which acts as active substrate for 5-enolpyruvylshikimate-3-phosphate synthase. Scarcity of the enzyme leads to the deficiency of aromatic amino acids, which affects various metabolic functions of the plant and hence destroys the plant (Tu et al. 2001).

Glyphosate translocation and uptake by plants

Glyphosate is promptly taken up by plant surfaces (Kirkwood et al. 2000). It is diffused through plant cuticle. The rate at which leaf imbibes glyphosate varies from species to species. Glyphosate has unique physicochemical properties due to which it is transferred from leaf, by means of phloem, to the tissues like roots, tubers and bulbs which are also the metabolic sinks of sucrose in plants (Siehl 1997). The phytotoxicity of glyphosate accomplishes meristems, storage organs, young roots, leaves and other growing tissues of the plant. Glyphosate shows its efficiency due to its excellent uptake by the plant, brilliant translocation to meristems, partial degradation and slow mode of action (Geiger et al. 1999).

Sorption of glyphosate in soil

When glyphosate combines with soil, that is if applied directly to the soil surface, released from plant roots or emitted from decomposed plant, it undergoes various chemical and physical changes which control its retention, transport and degradation. Retention of glyphosate in soil plays an important role, as it commands the accessibility of glyphosate for degradation, plant uptake and offsite transfer (Duke et al. 2012). Sorption of glyphosate to the soil is very large as compared to other pesticides, as it is a polyprotic molecule with three polar functional groups (amino, phosphate and carboxyl group) (Gill et al. 2017). Its sorption occurs on minerals, organic matter, variable charged surfaces (such as iron and aluminum oxides, aluminum silicates) and goethite. The rate of sorption increases with the increase in the surface area of the minerals and decreased pH (Duke et al. 2012). Once the glyphosate is sorbed in the soil, it is not easily desorbed. Desorption and sorption are inversely proportional to each other (Mamy and Barriuso 2007). Desorption rate of glyphosate from the soil is very less and depends on the type of soil. About 5–24% of initially sorbed glyphosate is desorbed and the rest remains bounded in the soil (Al-Rajab et al. 2008). Due to this, only a slight amount of glyphosate is left available in the soil for plant uptake, degradation and interaction with metal cations (Gill et al. 2017). Glyphosate molecule has many active donor sites, so it can form chelates and complexes with metal ions present in the soil. Cu and Zn ions get strongly complexed with glyphosate, whereas Fe, Ca, Mg and Mn ions get complexed to lesser amount (Vereecken 2005).

Degradation of glyphosate in soil

Glyphosate is degraded by microorganisms present in the soil. Biological degradation pathway involves the cleavage of glyphosate to glyoxylate and aminomethylphosphonic acid by the enzyme glyphosate oxidoreductase. It also gets degraded to methylamine and inorganic phosphate in the presence of enzyme C-P lyase. Further, both methylamine and glyoxylate are consumed by the microorganisms. Glyphosate can also be converted to aminomethylphosphonic acid and glyoxylate in the presence of glycine oxidase (Pollegioni et al. 2011). Another degradation pathway involves the cleavage of glyphosate to inorganic phosphate and sarcosine by enzyme C-P lyase. Sarcosine is further degraded to formaldehyde and glycine which are utilized by microorganisms present in soil (Dick and Quinn 1995). Glyphosate is strongly adsorbed by the soil, so its degradation by the microorganisms is quite slow. Its average half life in soil is 2 months. Degradation rate of glyphosate is affected by the type of microbial community present in the soil (Tu et al. 2001). It is easily degraded by the enzymes released by microbes, which help in the cleavage of C-P bond of glyphosate molecule. Similar type of metabolic processes was investigated in a Pseudomonas PG2982 strain that break glyphosate into phosphorous (Jacob et al. 1985; Moore et al. 1983). Other microorganisms like *Rhizobium meliloti*, Arthrobacter GLP-1 strain, *Agrobacterium radiobacter* and *Rhizobium* strains also show analogous pathway for glyphosate degradation (Dick and Quinn 1995; Liu et al. 1991; McAuliffe et al. 1990; Pipke et al. 1987). Another bacterial strain Arthrobacter GLP-1/Nit-1 utilizes glyphosate as nitrogen source (Pipke and Amrhein 1988). *Streptomyces spp.* consumes glyphosate for both phosphorus and nitrogen (Obojska et al. 1999). The presence of phosphorus in glyphosate is responsible for its microbial degradation, as phosphorus is required by the microorganisms for their metabolic functions (Lane et al. 2012).

Environmental profile of glyphosate

Glyphosate binds the soil constituents firmly; hence, it has no soil activity; thus it is foliar-applied and is post-emergent herbicide. It has very little seepage to groundwater and causes minimum contamination. It has fairly short half life, due to its degradation by the microorganisms. It is nonvolatile and does not contaminate atmosphere (Duke and Powles 2008). When used in recommended dose, glyphosate has little or no effect on nontarget organisms except some species of fungi (Franz et al. 1997). It is soluble in water and does not accrue in food web (Lane et al. 2012). Glyphosate changes the activity of various enzymes present in the soil. For example, it decreases the activity of enzyme phosphomonoesterase by 40–70%. In the case of urease and β-glucosidase, the activity was reduced by 5–40%, whereas for the enzyme dehydrogenase the activity was reduced up to 70% at soil pH 6.9. However, it does not affect the activity of enzymes (fluorescein diacetate hydrolase and arylsulfatase) present in soil at pH 6.6 (Riah et al. 2014).

Development of glyphosate-immune crops

Low animal toxicity and high herbicidal activity (Henderson et al. 2010) are the main aspects for the widespread use of glyphosate throughout the world (Sansom 2012; Steinmann et al. 2012; Garthwaite et al. 2010; CCM Information International report CCM International 2011). Excessive use of glyphosate in the farming led to the evolution

of glyphosate-resistant crops in 1996 (Johnson et al. 2009). Thereafter, glyphosate-resistant crops like cotton, soybean and corn were cultivated throughout the world (Duke et al. 2012). With the progression in the field of biotechnology, *Agrobacterium sp.* gene (CP4) was used to conceal glyphosate-resistant 5-enolpyruvylshikimate-3-phosphate synthase. Similarly genes from *Ochrobactrum anthropi* were used to assimilate glyphosate resistance in canola plants (Padgette et al. 1996). Also genetic mutations were performed on maize genes to introduce glyphosate resistance in maize plants (Vande Berg et al. 2008). With the commencement of these tailored transgenic plants in agriculture the use of glyphosate has increased manyfold. This resulted in the deterioration of farming systems. Also weed species had become more and more immune to this herbicide (Cerdeira et al. 2011). Consequently, it becomes intricate to stifle them. To overcome the rise of glyphosate-resistant weeds, genetically engineered crops were cultivated that were resistant to more than one kind of herbicide.

Evolution of glyphosate-resistant crops was a major breakthrough in the field of agriculture. Hence, the farmers became more casual and started using glyphosate with the loose hand. Such an over use of glyphosate resulted in the presence of glyphosate residues and its metabolite aminomethylphosphonic acid in many food crops at their harvesting as well as in their processed food (Myers et al. 2016). The overuse of glyphosate is not only causing the development of resistant crops, passing over of residues in food materials but is also creating a significant toxic impact over a wide plethora of organisms in the environment.

Various formulations of glyphosate are available in the market worldwide. Table 1 shows the list of different glyphosate formulations available in the market.

Toxicity of glyphosate over a wide range of organisms

Gratuitous use of glyphosate is not only distressing the weed species but is also causing severe threat to several other nontarget organisms found in the environment (Alliance 1996). It affects the growth and other metabolic functions of many unicellular as well as multicellular organisms found in both soil and water (http://www.national-toxic-encephalopathy-foundation.org/roundup.pdf).

Unicellular organisms

(a) *Euglena gracilis* Glyphosate also shows its adverse effects on many single-celled organisms like *Euglena gracilis*. It has been found that the use of glyphosate (at concentration 3×10^{-3} M) decreases the chlorophyll content from 21 to 69%. It also reduces photosynthesis and respiration at levels below 1.2×10^{-4} M by 20% (Richardson et al. 1979).

(b) Mycorrhizal fungal species Glyphosate reduced the radial growth of all ectomycorrhizal fungal species like (*Cenococcum geophilum* Fr., *Pisolithus tinctorius* (Pers.) Coker and Couch and *Hebeloma longicaudum* (Pers.)) at concentrations $\geq$ 1000 ppm, and their growth was completely inhibited at concentrations $\geq$ 5000 ppm. *Cenococcum geophilum* Fr. species was least sensitive to the glyphosate (Estok et al. 1989).

Toxic effects of glyphosate were also studied on some of the common mycorrhizal fungal species like *Hebeloma crustuliniforme*, *Laccaria laccata*, *Thelephora americana*, *T. terrestris* and *Suillus tomentosus*. It has been found that glyphosate reduced the growth of these fungal microorganisms at concentrations above 10 ppm (Chakravarty and Sidhu 1987).

(c) Rhizospheric microbial communities Glyphosate shows negative impact on the growth of certain rhizospheric microbial communities. Bacteria like *Fusarium*, fluorescent *pseudomonads*, Mn-transforming bacteria, and indoleacetic acid-producing bacteria present in the rhizosphere soils of soybean were treated with glyphosate. Glyphosate increased the profusion of *Fusarium spp.* while it reduced the profusion of fluorescent *pseudomonads*, Mn-reducing bacteria and indole acetic acid-producing rhizobacteria (Zobiole et al. 2011).

Another research group also studied the effects of glyphosate on soil rhizosphere-associated communities and found that the application of glyphosate increases the relative abundance of *proteobacteria* (particularly gammaproteobacteria). But the excessive use of glyphosate on glyphosate-resistant crops like corn and soybean decreases the relative abundance of *Acidobacteria*. Since *Acidobacteria* are also implicated in biogeochemical processes, the decline in the profusion of these bacteria could lead to considerable changes in nutrient status of the rhizosphere and would affect plant growth (Newman et al. 2016).

(d) Poultry microbiota Further the effects of glyphosate on some common pathogens and useful members of poultry microbiota were studied in vitro, and it had been found that extremely pathogenic bacteria like *Salmonella gallinarum*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Clostridium perfringens and Clostridium botulinum* are resistant to

Table 1 Common formulations of glyphosate used worldwide

S. No	Brand name	Active ingredients
1.	Roundup® Renew	360 g/L Glyphosate
2.	Watson weed killer 360 concentrate	Glyphosate isopropyl amine salt (360 g/L)
3.	McGregor's Weedout	Glyphosate isopropylamine salt (48%)
4.	Agpro Glyphosate 360	Glyphosate isopropylammonium salt (360 g/L)
5.	Dow Glyphosate 360	Glyphosate isopropylamine salt (360 g/L)
6.	Clearout 180	Glyphosate isopropyl ammonium salt
7.	Clearout 360	Glyphosate isopropyl ammonium salt
8.	Cobra	Glyphosate isopropyl ammonium salt
9.	Erase	Glyphosate isopropyl ammonium salt
10.	Glygran SG	Glyphosate ammonium salt
11.	Glyphofix	Glyphosate isopropyl ammonium salt
12.	Glyphogan	Glyphosate isopropyl ammonium salt
13.	Glyphosate WSG	Glyphosate-sodium
14.	Kalach	Glyphosate-sodium
15.	Mamba	Glyphosate isopropyl ammonium salt
16.	Mamba MAX	Glyphosate isopropyl ammonium salt
17.	Nexus Glyphosate	Glyphosate isopropyl ammonium salt
18.	Rondo	Glyphosate-sodium
19.	Roundup	Glyphosate isopropyl ammonium salt
20	Roundup Bio-dry	Glyphosate-sodium
21.	Roundup CT	Glyphosate isopropyl ammonium salt
22.	Roundup Turbo	Glyphosate isopropyl ammonium salt
23.	Roundup Ultra	Glyphosate isopropyl ammonium salt
24.	Slash Turbo	Glyphosate isopropyl ammonium salt
25.	Sting	Glyphosate isopropyl ammonium salt
26.	Touchdown	Glyphosate + trimesium
27.	Touchdown plus	Glyphosate + trimesium
28.	UAP Glyphosate	Glyphosate-sodium
29.	Back draft	Glyphosate + imazaquin
30.	Expert	Glyphosate + S-metolachlor + atrazine
31.	Extreme	Glyphosate + imazethapyr
32.	Flexstar GT	Glyphosate + fomesafen
33.	Sequence	Glyphosate + S-metolachlor

glyphosate. But the vulnerability of glyphosate toward the beneficial bacteria of poultry like *Enterococcus faecalis, Enterococcus faecium, Bacillus badius, Bifidobacterium adolescentis* and *Lactobacillus spp.* varies from species to species. Some of these microorganisms are highly susceptible, while some are moderate. Out of these, *Campylobacter spp.* (which are responsible for gastrointestinal diseases in humans) are highly prone to glyphosate. Intake of glyphosate by the poultry reduces the beneficial bacteria present in the gastrointestinal tract, which could perturb the normal functions of bacterial community present in the gut of these birds (Shehata et al. 2013).

(e) Periphyton communities Runaway of glyphosate from soil to water has also affected the unicellular organisms present in the periphyton communities present in the lakes and rivers. Goldsborough and brown found that glyphosate suppresses the photosynthetic rates in the lentic periphyton systems at concentrations of 89 and 890 mg/L (Goldsborough and brown 1988). Also, it stimulates certain structural changes in the diatom community present in lotic systems (Sullivan et al. 1981). Furthermore, glyphosate can act as potential phosphorus source for certain microorganisms, which could increase the detrimental eutrophication in water systems. This enhances the algal growth which could indirectly affect the aquatic life (Austin et al. 1991).

Multicellular organisms

Glyphosate's toxicity is not only observed in unicellular organisms but also shows its toxic effects on many multicellular organisms found on both soil and water.

(a) Algae Algae are diverse group of aquatic organisms that have ability to conduct photosynthesis. Wide varieties

of algae are found in aquatic systems. Glyphosate has also shown its toxic effects on various algal species.

Saenz et al. verified the toxicological effects of glyphosate and its commercial formulation Ron-do on two fresh water green algal species, *Scenedesmus acutus* and *Scenedesmus quadricauda*. It was found that the concentration of glyphosate in its commercial formulation (Ron-do) found in water systems does not cause acute toxicity to both these algal species. But this could cause long-term chronic and sublethal effects on *Scenedesmus quadricauda* population. Significant decrease in chlorophyll "a" content was also observed when *S. quadricauda* was exposed to 50 mg/L concentration. However, this herbicide does not signify any harmful chronic effects in the case of *Scenedesmus acutus* (Saenz et al. 1997).

Another research group also studied the effect of glyphosate on different macroalgae and seagrasses species of marine and anchialine aquatic systems (*Gayralia oxysperma, Rhizoclonium riparium, Ulva intestinalis, Pterocladiella capillacea, Myriophyllum aquaticum* and *Ruppia maritima*). Glyphosate reduces the chlorophyll content in the tested species even at the lowest concentration of 0.45 g L^{-1}. *Pterocladiella capillacea* showed maximum sensitivity, while *Gayralia oxysperma* was least sensitive toward this herbicide (Kittle and McDermid 2016).

Negative effects of glyphosate, Roundup and aminomethylphosphonic acid (main degradation product of glyphosate) on macroalgae (*Nitella microcarpa* var. *wrightii*) were studied by Oliveira et al. Three different concentrations (0.28, 3.5 and 6 mg/L) of glyphosate and Roundup were used. It has been found that glyphosate when applied in association with Roundup hampered the photosynthesis process. The rate of inhibition depends on the concentration of the herbicide used and the exposure time. In contrary to glyphosate, aminomethylphosphonic acid stimulates the photosynthesis process in algae. It degrades into phosphorus which provides nutrients to the algae. Excessive use of glyphosate-based herbicides affects both spatial and temporal distribution of *Nitella microcarpa* var. *wrightii* in the ecosystem (Oliveira et al. 2016).

Acute toxicity of glyphosate, isopropyl salt of glyphosate, Roundup (commercial formulation of glyphosate) and its common adjuvant polyoxyethyleneamine (POEA) on two common algal species *Selenastrum capricornutum* and *Skeletonema costatum* were checked by Tsui and Chu. It was found that out of these two algal species *S. costatum* is 7–10 times more sensitive than *S. capricornutum* to glyphosate either in acid form or in its salt form. The LC_{50} value at 96-h exposure is found to be 2.27 mg/L (glyphosate) and 5.89 mg/L (isopropyl salt of glyphosate) for *S. costatum* species, whereas LC_{50} values for *S. capricornutum* for glyphosate and its salt form are 24.7 and 41.0 mg/L, respectively. This variation in the LC_{50} values for these herbicides in both algal species may be due to phylogenic structural differences between them (Tsui and Chu 2003).

(b) Invertebrates Glyphosate showed its toxic effects on many invertebrates found on both land and water. No relevant data were found on the toxicity of glyphosate on lower invertebrates like protozoa, porifera, coelenterates and platyhelminthes.

(i) Nemathelminthes Nemathelminthes are an important class of lower invertebrates. They are mostly parasitic, but their abundance, diversity and correlation with soil indicate the state of the ecosystem. While some of the nematodes cause diseases, but they are also helpful in maintaining the earth's nutrient cycle and augment the assortment of natural ecosystem (Achiorno et al. 2008). Use of glyphosate in soil systems have also affected the nematodes and caused many toxic effects in them.

Achiorno et al. (2008) appraised the effect of various concentrations of glyphosate (both technical grade and formulation) on *Chordodes nobili*. Embryo, larvae and adults were used for performing the experiment. Test organisms were bared to all the concentrations ranging from 0.1 to 8 mg/L of glyphosate. Larvae hatched from the eggs treated with glyphosate ($\geq$ 0.1 mg/L) do not show any deformity in their development; however, their infective capacity was appreciably decreased. Comparable results were also achieved when larvae were directly exposed to the herbicide. Both the technical grade and formation of glyphosate presented similar kind of results. However, the adult worms exposed to 1.76 mg/L of glyphosate (for 96 h) showed 50% mortality (Achiorno et al. 2008).

(ii) Annelids: Earthworms Earthworms are the essential component of soil biota. They are helpful in maintaining the quality and ecosystem of soil (Datta et al. 2016). Various acute and chronic toxicity tests were conducted by different researchers to evaluate the toxicological effects of glyphosate on these wigglers.

Correia et al. conducted laboratory tests on *Eisenia fetida* to investigate the toxicological effects produced by glyphosate on it. Five different concentrations of glyphosate (1, 10, 100, 500 and 1000 mg) were used as test concentrations. The experiment was carried out for 56 days. No mortality was observed in the soils treated with glyphosate at any of these concentrations. However, steady and considerable decrease in the mean body weight was found at all the test concentrations. Glyphosate revealed severe toxic effects on the reproduction and development of earthworms in the range of test concentrations. No cocoons or juveniles were found in the soil treated with the herbicide. Apart from this, significant anatomical changes were also observed after 30 days of the experiment. Morphological abnormalities like the elevation

of body, coiling and curling were observed in all the specimens exposed to the highest test concentration of soil treated with glyphosate (Correia and Moreira 2010).

Another research group checked the acute and chronic toxicological effects of aminomethylphosphonic acid, the main metabolite of glyphosate on *Eisenia andrei* at field-relevant concentrations. No significant mortality was observed in both acute and chronic assays. In acute toxicity test, momentous loss in the biomass of earthworms was recorded in case of control as compared to the earthworms treated with aminomethylphosphonic acid. However, in chronic test, larger loss in the biomass of earthworms was recorded at the highest concentration of aminomethylphosphonic acid. Also there was an increase in the number of juveniles and cocoons at the highest concentration of the herbicide. But the mean body weight of these juveniles was found to be decreased. These results confirmed that juveniles are more sensitive to aminomethylphosphonic acid than the adults (Domínguez et al. 2016).

Ecotoxicological effects of glyphosate were evaluated on *Eisenia fetida* and *Eisenia andrei*. In the bioassays, earthworms were rendered to the soil samples collected from soya farms (treated with glyphosate), from Argentina. Both behavioral and biological changes were noticed in the test organisms of both species. It was observed that glyphosate decreased the cocoon viability, thereby decreasing the number of juveniles produced. Apart from this, they also avoided the soils treated with glyphosate and show reduction in their feeding activity (Casabe et al. 2007).

Similar kind of studies was conducted by Yasmin and D'Souza on *Eisenia fetida* to check the toxicological effects of glyphosate and other pesticides on it. A regular diminution in the body weight of the test organisms was found, when they were exposed to glyphosate and mixture of glyphosate, carbendazim and dimethoate (Yasmin and D'Souza 2007).

Hazardous effects of commonly used herbicide glyphosate on two annelid species *Eisenia fetida* and *Octolasion tyrtaeum* were studied by García-Torre et al. (2014). Both these test organisms were exposed to five different concentrations of glyphosate. Results reveal that earthworm species *Octolasion tyrtaeum* was more prone to the highest concentration of glyphosate (50,000 mg kg^{-1}). 100% mortality was observed at this concentration after seventh day of treatment. However, in the case of *Eisenia fetida* no mortality was recorded, but a noticeable loss (40%) in the body weight was found. Adverse effects of the herbicide were also found on the adult fecundity and cocoon viability. The number of juveniles produced from the cocoons was also decreased (García-Torre et al. 2014).

Berghausen et al. also assessed the impact of glyphosate-based herbicides on two species of earthworms (*Lumbricus terrestris* and *Aporrectodea caliginosa*). The surface casting activity of *Lumbricus terrestris* was decreased after three weeks of herbicide application. However, no change in this activity was recorded for other earthworm species (*Aporrectodea caliginosa*). Apart from this, reproduction rate in earthworms of both species was also reduced within 3 months after herbicide application (Gaupp-Berghausen et al. 2015).

Toxicity evaluation of two glyphosate-based herbicides was carried out by comparing their adverse effects on earthworm (*Eisenia andrei*). Glyphosate's commercial formulations, Roundup FG and Mon 8750, were used. Lethal concentration (LC-50) values reveal that Roundup FG was 4.5 times more toxic than Mon 8750. However, at sublethal concentrations noticeable weight loss was observed. Glyphosate acts as uncoupler of oxidative phosphorylation in the mitochondria of earthworms. Roundup FG showed venomous effects on the DNA of test organisms and caused lysosomal damage in them (Piola et al. 2013).

Sublethal effects on the population dynamics of earthworm species *Eisenia fetida* were carried out by Santadino et al. Two different concentrations of the herbicide were used. Glyphosate showed long- term effects on the test organisms with the decrease in the fertility of cocoons. This led to the local extinction of population of the earthworms in the soil (Santadino et al. 2014).

Assessment of the effect of the pesticide to the nontarget organisms present in the soil was done by Santos et al. Three commercial formulations containing insecticides (Chlorpyrifos, Endosulfan) and the herbicide (Glyphosate) were used. Treated soil was collected to verify the avoidance test and reproduction behavior of *Eisenia andrei*. These worms avoided the soil contaminated with Chlorpyrifos and Endosulfan. However, in the case of glyphosate, an equal number of worms were found on both sides indicating that glyphosate does not cause any harm to earthworms if used in recommended dose. Also it does not affect the reproduction activity of the worms (Santos et al. 2012).

Glyphosate molecule has many binding sites due to the presence of different functional groups present in it. It can easily combine with metal ions and form metal complexes. Fan Zhou et al. found that Cu ions present in the soil form complex with glyphosate and reduce the acute toxicity on earthworm caused by Cu ions. This complexation declined the mortality rate in earthworms. Along with this superoxide dismutase (SOD), glutathione (GSH) content and acetylcholinesterase activity were also reduced to the levels of control. These outcomes revealed that the complexation of glyphosate with metal ions present in soil could reduce the toxicity and accessibility of heavy metal ions present in the soil (Zhou et al. 2012).

Another research group used glyphosate-based herbicide Groundclear (containing 5% of isopropylamine salt of glyphosate), to examine its acute toxicity on *Eisenia fetida*. Earthworms were exposed to five different concentrations

of the herbicide; however, the worms exposed to the recommended dose for 24–48 h show very little mortality. But they show avoidance behavior against the herbicide. The presence of herbicide in the soil also affects the locomotor activity of the worms. Thus, the use of herbicide may not directly cause any harm to them, but it can cause severe long-term effects (Verrell and Van Buskirk 2004).

Zaller et al. analyzed the effects of glyphosate-based herbicide on the correlation between earthworms (*Lumbricus terrestris*) and symbiotic mycorrhizal fungi. Herbicide application on the soil decreased the earthworm activity in the mesocosms containing arbuscular mycorrhizal fungi. It further declined the soil mycorrhizal fungi spore biomass, vesicles and reduced the root mycorrhization. This resulted in the poor interactions between the worms and the mycorrhizal fungi which pose a serious threat to the natural systems (Zaller et al. 2014).

(iii) Arthropods Arthropods constitute 90% of the animal kingdom and are one of the biggest groups of invertebrates. They play an important role in maintaining ecological balance, provide livelihood and nutrition to human communities (Whiles and Charlton 2006). Extreme use of herbicides has many direct and indirect effects on them. Different research groups evaluated the impacts of glyphosate on arthropods as follows.

(iii(a)) Crustaceans Crustaceans are the very large group of arthropods found in freshwater and sea water. They form an important part of human diet. Apart from this, they are beneficial to the aquatic ecosystem as they help in the destruction and decaying of microscopic plants present in water. They themselves are eaten up by larger sea animals and maintain the balance of aquatic food chains. Leaching of glyphosate from soil to the water system has affected many aquatic animals and poses severe toxic impacts over them (Pérez et al. 2011). Toxicological impacts of glyphosate on sea animals were evaluated on Daphnia (as a test organism) by various researchers.

Daphnia Toxicity level of glyphosate (in its common formulation RON-DO) in water was checked on two planktonic crustacean species of daphnia. *Daphnia magna* and *D. spinulata* were exposed to glyphosate at different concentration (18, 32, 54, 90, 150 and 250 mg glyphosate a.i/L) for 24 and 48 h. It has been found that after 24-h exposure to the herbicide all the organisms of both the species become immobilized only at the highest concentration of 250 mg/L). And after 48-h exposure, immobility of organisms was found only at the concentration of 150 mg/L. These results showed that glyphosate is moderately toxic to *daphnia* (Alberdi et al. 1996).

Negative effects of glyphosate and its common formulation Roundup, on *Daphnia magna*, were also analyzed by another research group. They found that both glyphosate and Roundup are toxic to these aquatic invertebrates. Roundup proved somewhat lesser acute toxicity than pure glyphosate. The EC_{50} values of Roundup are 3.7–10.6 mg/L, while for glyphosate these values vary from 1.4 to 7.2 mg/L. However, Roundup was found to show more chronic toxicity in the tests spanning the complete life cycle of *Daphnia*. It has been found that at minimal test concentration of 0.05 mg/L, there occurs reduction of juvenile size. At 0.45 mg/L of Roundup, the growth, fertility and abortion rate were also affected. Significant negative effects were observed for both these herbicides at concentrations of 1.35 and 4.05 mg/L. Roundup at concentration of 1.35 mg/L showed 100% abortion of eggs. Also at this concentration, the embryonic stages are unfavorably affected (Cuhra et al. 2013).

The effect of common inorganic suspended sediment (Bentonite clay) on the acute toxicity of glyphosate on the aquatic organisms was checked by Hartman and Martin. *Daphnia pulex* was used to evaluate the toxicity level of the herbicide in aquatic systems. It has been established that the presence of suspended sediment increased the short-term toxicity of glyphosate at all the test concentrations. There was a significant decrease in the number of total population of *D. pulex* on exposure to Roundup at different concentrations (with both and without the suspended sediment). However, this reduction in the number of organisms was less in the case of glyphosate solutions without the suspended sediment. Glyphosate showed its selective toxicity to immature individuals, and this reduction in immature population was more when suspended sediments were used (Hartman and Martin 1984).

Toxicity of glyphosate and its commercial formulation Faena on fresh water invertebrate *Dapnia magna* was investigated by Dominguez-Cortinas et al. They found that both glyphosate and Faena are toxic to aquatic nontarget organisms. Faena is found to be 1.7-fold more toxic to *Daphnia Magna* than pure glyphosate. Glyphosate is an organophosphate pesticide and targets the esterases system of animals. It inhibits the activity of this enzyme and affects various metabolic functions in animals. EC_{50} values for glyphosate are 0.6 μM, 0.1 mg L^{-1} (Dominguez-Cortinas et al. 2008).

In another toxicity evaluation of glyphosate and its commercial formulation Roundup by Szekacs et al., two different populations of *Daphnia magna* were used. One was obtained from standard laboratory (originated from LAB Research Kft., Veszprem, Hungary) and other was wild species (collected in Pest County, Hungary). Roundup showed acute 48-hour LD_{50} values in 560–1700 μg/mL range on the laboratory species. However, the wild species was more sensitive toward Roundup and it was found to be twice times sensitive to this herbicide formulation. Toxicity tests on standard

laboratory species *of D. magna* showed that Roundup was 35 times more toxic than glyphosate (Szekacs et al. 2014).

Hyalella castroi Dutra et al. observed the effects of glyphosate's commercial formulation Roundup on the biochemical composition, Na^+/K^+ ATPase activity, levels of lipoperoxidation and reproductive traits in the freshwater amphipoda, *Hyalella castroi*. Test organisms were exposed to four different concentrations of the herbicide (0.36, 0.52, 1.08 and 2.16 mg/L). It was found that glyphosate exposure affected the reproductive activity of the animal. No mating pairs, ovigerous females or eggs in the marsupium of the matured females were observed at any of the tested concentration. Also the survival rate of the organism was lowered at all the concentrations (0.36 mg/L-73.92%, 0.52 mg/L- 61.12%, 1.08 mg/L-55.32% and 2.16 mg/L-47.72%). Glycogen level was also found to decrease in the tested organisms, and this decrease was dose dependent. But the decrease was more intense in males. Protein level was also decreased, and this decrease was again dose dependent. It was found that in the case of male *Hyalella castroi* the total protein content increased with the increase in the glyphosate concentration. Also the lipid level, triglycerides level, cholesterol content, lipoperoxidation and Na^+/K^+ ATPase activity were significantly reduced at all the test concentrations of glyphosate (Dutra et al. 2011).

Crayfish Effects of glyphosate and the common surfactant polyoxyethyleneamine (POEA) were verified on freshwater crayfish (*Cherax quadricarinatus*) by Frontera et al. Sublethal effects of 50-day exposure to glyphosate (22.5 mg/L), polyoxyethyleneamine (7.5 mg/L) and the mixture of both in the ratio of 3:1 were checked on the growth of the juvenile crayfish. Exposure of crayfish to the mixture of glyphosate and polyoxyethyleneamine resulted in lower somatic cell growth and decreased muscle protein level in the fish. It also reduced the muscle glycogen stores and lipid reserves of the fish. Also, an appreciable amount of weight loss was noticed in the fish when exposed to either of these glyphosate or the surfactant polyoxyethyleneamine (Frontera et al. 2011).

Glyphosate toxicity was checked on early juveniles of crayfish, by exposing them for 60 days to two test concentrations (10 and 40 mg/L). 33% mortality was perceived in the crayfish juveniles, exposured to highest concentration of the glyphosate. No significant divergence was noted in the molting process of the animal at any of the test concentrations. Decrease in the weight gain was recorded at both the glyphosate concentrations. However, a significant decrease was observed in the weight gain after first month of exposure at 40 mg/L of glyphosate. Apart from this a considerable decrease was found in the lipid level in muscles and protein level in both hepatopancreas and muscles of the crayfish (Avigliano et al. 2014).

(iii(b)) Insects Insecta is the largest group of invertebrates in phylum arthropoda. In terrestrial ecosystems, insects play a vital role as pollinators, herbivores, seeds dispersers, predators and detritivores. They work as ecosystem engineers.

Honey bee Excessive use of glyphosate has also affected the population of bees all over the world. It kills the potential food source for honey bees by destroying the non-crop plants. Apart from this, it exterminates the beneficial bacteria found in the gut of honey bees (Burlew 2010) and caused many toxicological effects on them. Toxicological impact of glyphosate on honey bees was evaluated by different researchers all over the world.

To analyze the toxicity level of insecticides and herbicides on insects, Boily et al. evaluate acetylcholinesterase activity in honey bees (*Apis mellifera*) by exposing them to maize treated with three different pesticides (neonicotinoids, glyphosate and acephate). Honey bees were revealed to sublethal concentrations of insecticide (neonicotinoids) and herbicides (glyphosate and acephate) under controlled conditions. Abnormal increase in the acetylcholinesterase level was found in the bees treated with neonicotinoids, while slight decrease was recorded in the case of glyphosate. (Boily et al. 2013).

Another research group checked the toxicological effects of glyphosate on honey bee (*Apis mellifera*) by analyzing their appetite behavior. Honey bees were exposed chronically and acutely to the recommended doses of glyphosate. Diminished sensitivity to nectar and poor learning performance were observed in the case of young adult bees treated chronically to the herbicide. However, in acute toxicity test performed at the recommended doses, decrease in the elemental learning and diminished short-term memory retention was examined. In spite of this, non-elemental associative learning was also damaged, though rummage for nectar was not affected by this herbicide. But a serious problem may arise when bees might carry nectar with glyphosate traces in it; this could distress the other nest mates and has all long negative consequences (Herbert et al. 2014).

Effects of sublethal concentrations of glyphosate on honeybee navigation were carefully investigated. Three different sublethal concentrations (2.5, 5.0 and 10 mg L^{-1}) of glyphosate were mixed with sucrose solutions, and bees were nourished with them. Steering path followed by the bees from the source of food to their hives was detected using harmonic radar system. It was observed that honey bees fed with sucrose solution containing highest dose of glyphosate took more time to come to their hives than the control bees. They also show more indirect flights to their nests. These results revealed that glyphosate impaired the intellective capability of bees, thereby making it difficult for them to return to their nests (Balbuena et al. 2015).

To study the effect of different pesticides on honey bees (*Apis mellifera*), larvae were treated with different concentrations. All the pesticides triggered the apoptosis in the treated larvae. The cell death was detected by DNA fragmentation labeling from midgut, salivary glands and ovaries of the treated larvae. 69% cell death was observed in the midgut of larvae treated with glyphosate (Gregorc and Ellis 2011).

Wasps Bueno et al. evaluated the pernicious nature of pesticides used in soybean crops on the *Trichogramma pretiosum*. Collection of different insecticides and herbicides was appraised, and varying results were obtained from different pesticides. Glyphosate-based herbicide (Roundup Ready) was found to be harmful for the eggs of the parasite but harmless for other parasitoidal stages. However, glyphosate's other formulation, Glyphosate 960 (Roundup original) was slightly harmful for eggs, but it remained harmless for pupae of the parasitoid (Bueno et al. 2008).

(iv) Molluscs Molluscs are considered to be ecological indicators, and their condition reflects the vigor of the entire ecosystem. They are the important food source for many aquatic animals and act as recyclers of plants and animal waste in aquatic systems. Seepage of glyphosate from agricultural lands has caused havoc to these tiny eco-friendly critters.

Snails Toxicity effects of glyphosate were also studied on different species of snails both aquatic and terrestrial by different groups of ecologists.

Tate et al. studied the long-term effects of glyphosate on the development and survival of aquatic snails (*Pseudosuccinea columella*) which act as intermediate host for liver fluke (*Fasciola hepatica*). Three successive generations of the snails were assessed to three different concentrations of glyphosate (0.1, 1.0, 10 mg/L) for 12 days. Glyphosate showed little effect on the first and second generations of snails. However, at concentration of 1.0 mg/L, the embryos of third generation developed much faster than other two concentrations. Hatching of eggs was repressed at the highest concentration of 10 mg/L and slightly inhibited at 0.1 mg/L. Snails exposed to the glyphosate concentrations of 0.1 and 10 mg/L showed an abnormal increase in egg-laying capacity and polyembryony in their eggs. This means that glyphosate affects the reproduction and development of snails (Tate et al. 1997).

Effect of sublethal concentration of glyphosate on the protein content and aminotransferase activity in *Pseudosuccinea columella* was checked by Christian et al. Three different concentrations of glyphosate were used (0.1, 1.0 and 10 mg/L). Results showed a significant increase in the aminotransferase/glutamic oxaloacetic transaminase activity in all the snails reared at all the test concentrations of glyphosate. However, alanine aminotransferase/glutamic pyruvic transaminase activity was decreased. Also there was an unequal enhancement in the aminotransferase activity in the body fluids of the snails exposed to glyphosate. Apart from this, slight decrease was observed in the aminotransferase activity/glutamic oxaloacetic transaminase/glutamic pyruvic transaminase in the snails cultured at glyphosate concentrations of 0.1 and 10 mg/L. But the most significant results were shown at the lowest test concentration (0.1 mg/L), which specified that the lower concentrations of glyphosate were more easily absorbed and metabolized by the test organism. Also it was found that over an extended period of time, there comes a saturation of the toxification in an organism at about 1 mg/L for glyphosate. This proved the lack of consistent dose-dependent effects with in glyphosate concentration in the snails (Christian et al. 1993).

Assessment of the long-term effects of glyphosate on the terrestrial snail species *Helix aspersa* was done by Druart et al. Newly hatched snails were exposure to the soil and food contaminated with the herbicide for 168 days. Recommended field dose and tenfold the recommended dose of glyphosate were used for the experiment. No effects on the survival and growth of the snail were observed. But the presence of glyphosate was detected in the tissues of the snails which were continuously fed on the food contaminated with the herbicide. This concluded that there will be risk of transfer of glyphosate to food chain (Druart et al. 2011).

(v) Echinoderms Echinoderms are the important invertebrates that play numerous roles in ecological balance. They burrow deep in the sand and provide more oxygen at greater depths of sea floor. Some of the echinoderms like starfish prevent the growth of algae on coral reefs. Glyphosate has proved risky effects on these sea creatures and has caused devastation to them.

Sea Urchins To comprehend the injurious effects of the commonly used pesticide, sea urchins were used as the model animals by Marc et al. The pervasive use of glyphosate herbicides has influenced the early development in sea urchins and hampered their hatching process. Along with this, the active surfactant polyoxyethylene amine was also found to be highly noxious to the embryos. This illustrated that these herbicides have impinged the transcription process in sea urchins and affected their health (Marc et al. 2005).

Alterations caused by glyphosate-based herbicides in the cell division of sea urchins were studied by Marc et al. Different glyphosate formulations with different concentrations were used, and it was established that glyphosate at the highest concentration of 2 mM detained the cell cycle in these

organisms and the embryos developed into unhealthy adults (Marc et al. 2004).

Table 2 shows the toxic effects of glyphosate on various species of invertebrates.

(c) Vertebrates Toxic effects of glyphosate were also studied on different classes of vertebrates.

(i) Fish Fish is one of the most important classes of chordates in aquatic ecosystems. Fish are an integral part of natural ecosystem and provide immense economic, ecological and cultural values through food fishing. Fish forms an important part of food chain and maintains natural balance of food webs. Different researchers from all round the world evaluated the toxic impacts of glyphosate on different types of fish.

Acute and subacute toxic effects of sublethal glyphosate in water were studied by Neskovic et al. on fresh water fish carp (*Cyprinus carpio* L.) Toxicity test were performed on three different concentrations of glyphosate (2.5, 5 and 10 mg/L). LC_{50} values of glyphosate for the fish after 48- and 96-h exposure were 645 and 620 mg/L, respectively. This shows that glyphosate is slightly toxic to carp. Biochemical analysis on the liver, heart, kidneys and serum of the fish was done to check the noxious effects of glyphosate. An increase in the alkaline phosphatase activity was observed in the liver of the fish at all the test concentrations. At 10 mg/L of glyphosate, an increase in the alkaline phosphatase activity was recorded in the heart of the fish. Apart from this, at concentrations of 2.5 and 5 mg/L there was an increase in the glutamic oxaloacetic transaminase activity in the liver and kidneys. Also, an increase in glutamic pyruvic transaminase activity in the serum of the fish was recorded at 5 and 10 mg/L of glyphosate. Histopathological studies of glyphosate on carp showed that on exposure to 5 mg/L, the gills of fish developed epithelial hyperplasia and subepithelial edema. Similar changes were observed at concentration of 10 mg/L, but the results were more pronounced. Leukocyte infiltration, slight hypertrophy of chloride cell, lifting and rupture of the respiratory epithelium were also observed on some secondary lamellae. Apart from this, at glyphosate concentration of 10 mg/L congestion of sinusoids and early signs of fibrosis were also recorded (Neskovic et al. 1996).

Adverse effects and global mechanism of toxicity of glyphosate and its common formulation Roundup on brown trout (*Salmo trutta*) were analyzed by using RNA-sequencing by Webster and Santos. Juvenile female brown trout was exposed to different concentrations (0, 0.01, 0.5 and 10 mg/L) of glyphosate and Roundup for 14 days. Transcriptional profiling showed that both glyphosate and Roundup caused many variations in the complex interacting signaling pathways that control cellular stress response particularly in apoptosis. It was found that at all the three test concentrations of Roundup and at the lowest concentration of glyphosate there was a common mechanism of toxicity and cellular response. Also both these herbicides increase cell proliferation and cellular turnover and an up-regulation of metabolic processes (Webster and Santos 2015).

Another research group Murussi et al. investigate the effect of three different formulations of glyphosate 48% (Orium®, Original® and Biocarp®) on silver catfish (*Rhamdia quelen*) at different concentrations (0.0, 2.5 and 5.0 mg/L) for 96 h. Enzymological studies were conducted on the liver and plasma of the fish, and certain alterations were observed in the enzymatic activity. Thiobarbituric acid-reactive substances were found to increase and the amount of catalase produced in the liver was decreased in all the treatments at all the test concentrations. Superoxide dismutase activity was increased at 2.5 mg/L concentration of Orium® and Original®. Its activity was also found to increase at 5 mg/L concentration of Orium® and Biocarp®. Glutathione-S-transferase activity was increased at 2.5 mg/L concentration of Orium® and decreased at the same concentration of Biocarp® in comparison with the control. Analysis of plasma also recorded certain alterations in the enzymatic processes of the fish. Alanine aminotransferase was decreased after exposure of fish to 2.5 mg/L concentration of Biocarp®. Similarly the amount of aspartate aminotransferase increased at 2.5 mg/L of Orium® and Original® and 5.0 mg/L of Biocarp® in comparison with the control. Histopathological studies on the liver of fish showed certain changes in hepatic tissue. vacuolization, leukocyte infiltration, the degradation of cytoplasm and melanomacrophage were noticed in the hepatocytes of the fish (Murussi et al. 2016).

Toxicity evaluation of glyphosate on acetylcholinesterase activity in the fish species *Cnesterodon decemmaculatus* was done by Helman et al. Three sublethal concentrations of glyphosate (1, 17.5 and 35 mg/L) were used. It was found that the fish remains alive even at the highest concentration of 35 mg/L. Inhibitory effects on the activity of acetylcholinesterase were observed in the anterior body section of the fish. The inhibition ranged between 23 and 36%. Decrease in the acetylcholinesterase activity was recorded in the anterior and middle body sections, but no change in the activity of this enzyme in the posterior part of the fish was recorded. This showed that acetylcholinesterase presented different sensitivity to glyphosate depending upon the enzyme location in the body (Menéndez-Helman et al. 2012).

Another research group Salbego et al. determined the toxic effects of glyphosate formulation Roundup on piava fish (*Leporinus obtusidens*). The fish was exposed to three different concentrations of Roundup (0, 1 and 5 mg/L) for 90 days. It was found that the acetylcholinesterase activity in the brain of the fish was decreased when the fish was exposed to Roundup concentration of 5 mg/L. Liver

Table 2 Effect of glyphosate on invertebrates

Phylum	Species	Exposure time	Response	References
Nemathelminthes	*Chordodes nobili*	96 h	Decrease in the infective capacity of larvae derived from exposed eggs, 50% Mortality at concentrations > 1.76 mg/L	Achiorno et al. (2008)
Annelids	*Eisenia fetida*	56 days	Reduction in mean bodyweight, no cocoons and juveniles, morphological abnormalities like the elevation of body, coiling and curling	Correia and Moreira (2010)
	Eisenia fetida and *Eisenia andrei*	28 days	Decreased cocoon viability and earthworms avoided the soil containing glyphosate	Casabe et al. (2007)
	Eisenia fetida	28 days	Regular diminution in the body weight	Yasmin and D'Souza (2007)
	Eisenia fetida and *Octolasion tyrtaeum*	28 days	100% mortality (*Octolasion tyrtaeum* at 50,000 mg/kg) weight loss, decreased cocoon viability and reduced fecundity	García-Torre et al. (2014)
	Lumbricus terrestris and *Aporrectodea caliginosa*	32 days	Decrease in surface casting activity and reduced rate of reproduction	Gaupp-Berghausen et al. (2015)
	Eisenia andrei	72 h	Weight loss, DNA damage and lysosomal damage	Piola et al. (2013)
	Eisenia fetida	40 days	Decrease in the fertility of cocoons	Santadino et al. (2014)
	Eisenia fetida	124 h	Affect locomotor activity and worms show avoidance behavior	Verrell and Van Buskirk (2004)
	Lumbricus terrestris	14 days	Declined soil mycorrhizal fungi spores biomass, poor interactions between worms and mycorrhizal fungi	Zaller et al. (2014)
Arthropods (Crustaceans)	*Daphnia magna* and *D. spinulata*	48 h	Organisms of both the species become immobilized	Alberdi et al. (1996)
	Daphnia magna	55 days	Reduction in juvenile size, growth rate decreased, fertility rate was affected and abortion rate was increased	Cuhra et al. (2013)
	Daphnia pulex	48 h	Decrease in the number of total population	Hartman and Martin (1984)
	Daphnia magna	48 h	It targets the esterases system of animals and inhibits the activity of this enzyme and affects various metabolic functions in animals	Dominguez-Cortinas et al. (2008)
	Hyalella castroi	7 days	No mating pairs, no ovigerous females and no eggs. Survival rate was decreased, and glycogen, protein, lipid, cholesterol and triglycerides level were also decreased. Lipo peroxidation and Na^+/K^+/ATPase activity decrease	Dutra et al. (2011)
	Cherax quadricarinatus	50 days	Decreased somatic cell growth, decreased muscle protein, reduced muscle glycogen, reduced level of lipids and loss in weight	Frontera et al. (2011)
	Cherax quadricarinatus	60 days	33% mortality of juveniles, decrease in weight gain, decrease in lipid level in muscles and decrease in protein level in heptopancreas and muscles	Avigliano et al. (2014)

Table 2 (continued)

Phylum	Species	Exposure time	Response	References
Arthropods (Insecta)	*Apis mellifera*	14 days	Abnormal decrease in acetylcholinesterase level	Böily et al. (2013)
	Apis mellifera	Prolonged exposure	Reduced sensitivity to nector, poor learning performance, diminished short-term memory retention, damaged non-elemental associative learning	Herbert et al. (2014)
	Apis mellifera	1 h	Impaired intellectual capability of bees; they become more wayward in their flights back home to their hives	Balbuena et al. (2015)
	Apis mellifera	4 days	69% cell death in the midgut of treated larvae	Gregorc and Ellis (2011)
	Trichogramma pretiosum	72 h (egg), 144 h (larvae), 192 h (pupae)	Harmful for eggs	Bueno et al. (2008)
Mollusca	*Pseudosuccinea columella*	12 days	Delayed hatchings of eggs, abnormal increase in egg-laying capacity and polyembryony in cells	Tate et al. (1997)
	Pseudosuccinea columella	4 weeks	Increase in aminotransferase/glutamic oxaloacetic transaminase activity, decrease in alanine aminotransferase/glutamic pyruvic transaminase activity, unequal enhancement in the aminotransferase activity in the body fluids	Christian et al. (1993)
	Helix aspersa	168 days	Glyphosate residues were present in the tissues of snails	Druart et al. (2011)
Echinoderms	*Sphaerechinus granularis*	–	Early development in the organism, hampered hatching process, invade the transcription process	Marc et al. (2005)
	Sphaerechinus granularis	–	Detained cell cycle, embryos develop into unhealthy adults	Marc et al. (2004)

glycogen content was decreased at both the round up concentrations. Also the hepatic glucose content was reduced when the fish was exposed to 5 mg/L of Roundup. Lactate levels in both liver and muscles of the fish were increased at both the Roundup concentrations. However, hepatic protein content remained constant at 1 mg/L but increased at 5 mg/L concentration of Roundup. In muscles, protein content has decreased with the increase in concentration of Roundup (Salbego et al. 2010).

Toxicity effects of Roundup on another fish *Jenynsia multidentata* were studied by Hued et al. (2012). Fish were exposed to 5, 10, 20, 35, 60 and 100 mg/L of Roundup concentrations. The LC_{50} was found to be 19.02 mg/L at 96-h exposure for both male and female. All fish exposed to higher concentrations (60 and 100 mg/L) died during the exposure period. Sexual activity of male fish was also reduced when exposed to 0.5 mg/L Roundup for 7 and 28 days. Fish exposed to 5 mg/L concentration presented lifting of secondary lamellar epithelium, edema formation and hypertrophy of epithelial cells. Also, at this concentration there occurred hydropic degeneration in the liver of the fish. At concentration of 10 mg/L, lifting of secondary lamellar epithelium and hypertrophy of chloride cells were observed in the fish while hydropic degeneration accompanied by blood sinusoid dilation and foci of leukocyte infiltration occurred in the liver. At 20 mg/L of Roundup, hypertrophy of chloride cells and slight thickening of secondary lamellae were observed in the gills of the fish and in the liver focal necrosis and infiltration of leukocytes along with blood sinusoid dilation and vascular congestion were observed. At concentration of 35 mg/L, there occur more pronounced mucous cell proliferation and severe hyperplasia of epithelium cells in the gills of the fish. As the concentration of the herbicide increases, more severe effects were observed in the fish (Hued et al. 2012).

Another research group also studied the toxic effects of glyphosate (Roundup) on the fresh water fish surubim (*Pseudoplatystoma*). Different concentrations of Roundup (0, 2.25, 4.5, 7.5 and 15 mg/L) were used to test the metabolic and behavioral changes in the fish after the exposure of 96 h. Glyphosate exposure altered the glucose level in the plasma of the fish. It reduced the level of glucose in the plasma, but increased it in the liver of the fish. Also the lactate level in both plasma and liver was increased, but it gets reduced in the muscles. Protein and glycogen levels were decreased in both plasma and muscles. Cholesterol was also decreased in the plasma of the fish at all the test concentrations. Apart from the metabolic alterations, certain changes were also noticed in the enzymatic activity of the fish. Alanineaminotransferase was found to increase in the plasma, but no significant change was noticed in the case of aspartate aminotransferase levels. Certain behavioral changes were also noticed in the fish. The ventilatory frequency was increased after glyphosate exposure for 5 min, but it finally gets decreased after 96-h exposure. Also the swimming activity of the fish was altered at the test concentration of 7.5 mg/L (Sinhorin et al. 2014). Mutagenic and genotoxic effects of glyphosate's formulation Roundup were studied by exposing poecilia reticulate for micronucleus test, comets assay and nuclear abnormalities. The fish were exposed to 0, 1.41, 2.83, 4.24 and 5.65 µL/L of the herbicide for 24 h. On analysis it was found that the number of micronucleus and comets had increased in the gill erythrocyte cells. This indicates that herbicide's exposure resulted in the increase in the number of damaged cells. Also the concentration of the herbicide used and the damage caused are positively co-related with each other (De Souza Filho et al. 2013).

Toxicity of the technical grade glyphosate, isopropylamine salt of glyphosate, formulated herbicide Roundup and Roundup surfactant was checked on four different fresh water fish, rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*) and bluegills (*Lepomis macrochirus*) by Folmar et al. Acute toxicity test showed that the LC50 value at 96 h for Roundup varied from one species of fish to other species. It was 2.3 mg/L for fathead minnow and 140 mg/L for the rainbow trout. Toxicity of surfactants was found to be similar to that of Roundup formulations. However, Roundup was found to be more toxic at higher temperature and at pH of 7.5 for rainbow trout and bluegills. But toxicity does not changes with the increase in pH value. Toxicity effects of the herbicide depend upon the life cycle of the fish. Eyed eggs were in the least sensitive stage, while sac fry and early swim-up stages are prone to glyphosate. Reproduction and gonadosomatic index in adult rainbow trout were not affected by this herbicide (Folmar et al. 1979).

Cattaneo et al. analyzed the effects of commercial glyphosate's formulation Roundup, on the activity of acetylcholinesterase enzyme and oxidative stress in Cyprinus carpio. Five different concentrations of the herbicide were used (0, 0.5, 2.5, 5 and 10 mg/L), and the fish was exposed to these concentrations for 96 h. It was found that after exposure, the acetylcholinesterase activity was repressed in the brain and muscle of the fish. Oxidative stress produced by the Roundup was measured by the amount of thiobarbituric acid-reactive species present in the fish. After exposure, an increase in thiobarbituric acid-reactive species was observed at all the test concentrations of the herbicide. These results confirmed the lipid peroxidation and anti-acetylcholinesterase action, stimulated by the Roundup on the fish (Cattaneo et al. 2011).

(ii) Amphibians Amphibians play an essential role in ecosystem as secondary consumers in both aquatic and terrestrial habitats and maintain balance in many food chains. Excessive use of pesticides has adverse effects on them. Decline

in amphibian population will have large-scale everlasting effects on ecosystem; it will boost up algal population and will affect primary production in the ecosystem (Whiles et al. 2006). Toxic nature of glyphosate and its adverse effects on frogs and toads were studied by different research groups.

Frogs Appraisal of venomous nature of glyphosate and its commercial formulation Roundup on the early developmental stages of frog (*Leptodactylus latrans*) was deliberated by Bach et al.

A broad range of concentrations varying from (0.0007–9.62 mg of acid equivalent per liter) of Roundup and (3–300 mg/L) of glyphosate were used. Larvae were fed with blended lettuce regularly till they reached the developmental stages Gosner stage 25 and 36. In the case of larvae fed with glyphosate, no mortal effects were monitored during any of these stages. However, Roundup affected the swimming and other morphological activities of the tadpoles. Oral abnormalities and edema were the common symptoms of herbicidal poisoning. Commercial formulation Roundup was found to be more toxic to frogs (Bach et al. 2016).

Noxious effects of glyphosate on the hepatic tissues and erythrocytes of frog species *Leptodactylus latinasus* were evaluated. Anurans were exposed to three different concentrations of glyphosate (100, 1000, 10,000 μg g^{-1}). Blood samples were collected from the frogs and were investigated for any malfunctioning caused by the herbicide in them. Use of glyphosate enhanced the melanin area in melanomacrophage clusters of the frogs. It also altered the presence of hepatic catabolism pigments into melanomacrophages along with the alterations in the nucleus of erythrocytes (Pérez-Iglesias et al. 2016).

Acute toxicity of a mixture of two herbicides dicamba and glyphosate was verified on the late developmental stage of *Rhinella arenarum* larvae. Both these herbicides have capability to interact with the DNA present in the blood stream of the frog. Genotoxicity of these herbicides was verified by the breaking of DNA strands which cause lesions in the peripheral blood cell of the frog when exposed for 96 h. Mixture of the herbicides induces cell damage to much higher frequency than when applied alone. Glyphosate synergically increases the toxicity of dicamba when used together (Soloneski et al. 2016).

A very authentic and reliable method was used by Lajmanovich et al. to evaluate the injurious effects caused by dermal uptake, of organophosphate pesticides (2,4-D, chlorpyrifos and glyphosate) on the common toad *Rhinella arenarum*. Toads were exposed to nominal concentrations of all the pesticides. Results confirmed that toad's exposure to these pesticides endured neurotoxicity, oxidative stress and immunological depression (Lajmanovich et al. 2015).

Pernicious effects of glyphosate and its other commercial formulations were evaluated on adult frogs and tadpoles of four different species (*Crinia insignifera, Heleioporus eyrei, Limnodynastes dorsalis,* and *Litoria moorei*) found locally in southwestern Australia. The toxicity tests were conducted for 48 h. Glyphosate isopropylamine was found to be non-toxic to the tadpoles, and no mortality was recorded at concentrations between 503 and 684 mg/L. However, technical grade of glyphosate was found to be toxic. The toxicity was due to acid intolerance of tadpoles toward glyphosate. However, the tadpoles of species *Litoria moorei* were showing greater sensitivity toward the herbicides. It was also established that the adults and newly evolved metamorphs were less sensitive than tadpoles toward the toxicity of the herbicides (Mann and Bidwell 1999).

Dornelles et al. investigated the pernicious effects of the common herbicides (atrazine, glyphosate and quinclorac) on the endurance and biochemical changes in the blood and body of bull frog tadpoles (*Lithobates catesbeianus*). Exposure to glyphosate resulted in a significant reduction in the glycogen and triglyceride level in all the organs of tadpoles. Along with this, an increase in lipid peroxidation and cholesterol level in the gills of the tadpoles was observed. However, muscles and total protein content in the gills were decreased. These alterations in the biochemical parameters of tadpoles showed the toxicity produced by the herbicides on the tadpoles (Dornelles and Oliveira 2016).

To evaluate the pestilential effects of glyphosate on frogs, another group of researchers exposed tadpoles of species *Rhinella arenarum* to four different commercial formulations of glyphosate (Round Ultra- Max, Infosato, Glifoglex and C-K YUYOS FAV). Tadpoles were treated with eight different concentrations of all the herbicides for 48 h. Results exhibited the consequential decrease in the activity of the main enzymes (acetylcholinesterase, butyrylcholinesterase, carboxylesterase and glutathione S-transferase) used in the catalyses of neurotransmitters (Lajmanovich et al. 2011).

Acute and chronical toxicological studies of glyphosate-based herbicides containing surfactant polyethoxylated tallowamine (POEA) were carried out by Howe et al. Four North American amphibian species ((*Rana clamitans*, *R. pipiens*, *R. sylvatica* and *Bufo americanus*) were used for toxicity analysis. Evaluation between the amphibian species demonstrated that the toxic level of the tested herbicides varied from species to species. However, *Rana pipiens* when exposed to the recommended dose of POEA or any glyphosate-based herbicide containing POEA showed adverse effects with the decreased snout-vent length at metamorphosis, tail damage with gonadal abnormalities. It also resulted in delayed metamorphosis. These results concluded that the surfactant POEA along with glyphosate is posing greater threat to the nontarget organisms (Howe et al. 2004).

(iii) Reptiles Reptiles play an essential role both as predators and as prey species. They maintain the ecological balance of most of the food webs. Devastating effects of glyphosate on the reptiles was examined by a number of research groups.

Crocodiles Natural habitats of crocodiles are found in the vicinity of agricultural lands, so the application of various herbicides and pesticides in the fields has noxious effect on the growth and development of them.

Genotoxicity and effects on growth of glyphosate-based herbicide (Roundup) on crocodilian species (*Caiman latirostris*) were evaluated by Gonzalez et al. Twenty-day-old hatchings of *Caiman latirostris* were exposed to two field recommended doses of Roundup for 2 months. At the end of the experiment, blood samples were collected from the test organisms and micronucleus test was applied in erythrocytes. Significant increase in the frequency of micronucleus was found in the samples. This indicated an increased DNA damage in the cells which may result in retarded growth in the organisms (Gonzalez et al. 2013). Another similar type of work was done by Poletta et al. to determine the genotoxic effects of Roundup in the erythrocytes of *Caiman latirostris, in ovo* treatment. Early Caiman embryos were exposed to different sublethal concentrations of Roundup. At the time of hatching, blood samples were collected from each organism and two short-term tests (the comet assay and micronucleus tests) were performed. Results showed a significant increase in DNA damage at higher concentrations of the herbicide (Poletta et al. 2009).

Distressing effects due to the overuse of glyphosate on nontarget organisms were appraised by Latorre et al. (2013) on crocodilian species *Caiman latirostris*. Twenty-day-old crocodiles were exposed to two different concentrations of glyphosate for 2 months. They were weighed before and after the test period. Blood samples from the exposed crocodiles were taken to substantiate the total protein content and total WBC count. Results confirmed the decrease in total WBC count with increase in the percentage of heterophils and total protein content. It also showed the negative impact on the growth of the organisms. Thus, in vivo exposure of the reptiles to this herbicide altered their immune system (Latorre et al. 2013).

Another research group also evaluated the hazardous effects of Roundup on the immune system of *Caiman latirostris*. Test organisms were exposed to two different concentrations of the herbicide (recommended doses for the field application). After the exposure time (2 months), WBC count and complement system activity were checked. The crocodiles were then injected with a solution of lipopolysaccharide from *E. Coli* to activate the immune response and to appraise the parameters associated with it. A momentous decrease in the activity of the complement system was observed along with the decrease in heterophils and lymphocytes count (Siroski et al. 2016).

Lizards Lizards are an important part of ecosystem. They help in maintaining ecological balance in food chain by thwarting the overpopulation of lower organisms. They are less imperative to humans but are significant for the complete food web. The common habitats of lizards are affected by the use of agricultural practices. Overuse of herbicides and pesticides also has negative impacts on these organisms.

Genotoxicity of the most commonly used herbicide Roundup on tegu lizard (*Salvator merianae*) was established by Schaumburg et al. *In ovo* treatment with six different sublethal concentrations of the herbicide was performed on the lizards. Blood samples were collected for micronucleus test, nuclear abnormalities and comet assay. Any external structural abnormality was also evaluated. Outcomes of the study revealed that the most commonly used herbicide has genotoxicity effects on tegu lizard. DNA damage was observed on the erythrocytes of the reptile after exposure to sublethal concentrations which may impede the developmental process of the neonates (Schaumburg et al. 2016).

An ecotoxicological study for the negative impact of glyphosate-based herbicides on common skink (*Oligosoma polychroma*) was carried out by Carpenter et al. Test organisms were exposed to two different formulations (Agpro Glyphosate 360, Yates Roundup Weed killer) at precise dose (144 mg/L of water) for 4 weeks. The lizards were confined during the experiment period for preferred temperature and mass. Any kind of change in the performance and behavior of lizards was noticed. None of these glyphosate formulations had any considerable effect on the mass of the organisms. Conversely, the lizards exposed to Yates Roundup Weed killer develop higher temperature to increase their metabolism and to thwart the physiological stress caused by herbicidal exposure (Carpenter et al. 2016).

(iv) Birds Birds are an important part of ecosystem and maintain balance in various food chains.Several studies have shown that the use of glyphosate in proposed extent does not cause any toxicological effect to many birds. However, their presence is affected by the glyphosate-treated areas due to non-availability of food and shelter (Forest info.ca).

Effect of use of glyphosate on wetland's vegetation which affected the densities of territorial male Red-winged Blackbirds (*Agelaius phoeniceus*), Marsh Wrens (*Cistothorus palustris*) and Yellow-headed Blackbirds (*Xanthocephalus xanthocephalus*) was appraised by Linz et al. Wetland containing cattail was randomly selected with 0% glyphosate (as control) and treated wetland as 50, 70 and 90% areal spray coverage of glyphosate. After 2 years of treatment, the densities of the birds were evaluated. The densities of Red-winged Blackbirds were higher in the wetland where no

glyphosate was sprayed. Similarly the abundance of Yellow-headed Blackbirds and Wrens was also found to be more in untreated wetlands as compared to the treated wetlands. This illustrates that the use of glyphosate reduced the wetlands vegetation which further affected the bird population (Linz et al. 1996).

Santillo et al. examined the effect of glyphosate-treated and untreated clearcuts on the breeding bird population. Results showed that the clearcuts treated with glyphosate had decreased complexity as compared to the untreated clearcuts. Therefore, the availability of total bird population was less in case of glyphosate-treated clearcuts. Birds like common yellowthroats (*Geothlypis trichas*), Lincoln's sparrows (*Melospiza lincolnii*) and alder flycatchers (*Empidonax alnorum*) were fewer in treated areas. These results exemplify that the use of glyphosate affected the versatility of the birds and reduced their natural habitats that affect their breeding population (Santillo et al. 1989).

Deleterious effects of glyphosate-based herbicide Roundup on the reproductive system of drake *Anas platyrhynchos* were analyzed by in vivo studies. Disclosure to the herbicide has affected androgen and estrogen synthesis which causes severe damage to the reproductive system of the male bird. It also resulted in the variation in the morphology of the testis and epididymal region, thereby affecting the male genital organs of the bird. All these effects were dose dependent and severely affect the reproduction of the species (Oliveira et al. 2007).

(v) Mammals Mammals are placed at the top most position in hierarchy of kingdom animalia. They play a crucial role for maintaining services and functions for the balance in ecosystem. Excessive use of glyphosate has also caused several toxic effects on lower and higher mammals. Different studies have been conducted to investigate the lethal effects of glyphosate on these organisms.

Rats Long-term toxic impacts of glyphosate and its salutary effects with zinc were determined by the histopathological changes that occur in the stomach, kidney, liver, brain, spleen and pancreas of the rats by Tizhe et al. The rats were firstly pretreated with zinc (with dose of 50 mg/kg body weight) and then were exposed to two different concentrations of glyphosate (14.4 and 375 mg/kg bodyweight) for 2 months. Some rats were exposed to the herbicide after their treatment with zinc. Severe histopathological changes were observed in the case of rodents exposed to higher concentration of glyphosate. Mucosal epithelial cells were deteriorated along with the degeneration of hepatic cells. Glomerular degeneration, mononuclear cells infiltration and tubular necrosis were noticed in the kidneys of the rats. Pancreas and spleen also got damaged, while no pathological changes were noticed in the rats supplemented with zinc (Tizhe et al. 2014a). Tizhe et al. in another work assessed the subchronic toxic effects of glyphosate and its supplemented effect with zinc on the hepatic and renal functions of Wistar rats. Rats were orally fed for 8 weeks with different concentrations of glyphosate and zinc. At the end of the study, blood samples were collected and assayed for total protein content, albumin, alanine, aspartate aminotransferase, alkaline phosphates and other ions present in the blood. From the results, it was concluded that exposure of rats to glyphosate caused both renal and hepatic toxicity which was alleviated by the presence of zinc with it (Tizhe et al. 2014b).

Another research group tested the effects of glyphosate and the common surfactant polyoxyethyleneamine used in herbicide Roundup on the reproductive system of male and female offsprings of Wistar rats. Lactating parent and pregnant female rats were orally fed with different concentrations of glyphosate (50, 150, 450 mg/kg). Results showed that glyphosate does not generate any maternal toxicity to female rats; only a delay in the vaginal canal opening was noticed. However, it affects the male reproductive system by decreasing the total sperm content in the adult male rats. Moreover, an increase in the number of abnormal sperms and a decrease in serum testosterone level were also observed in male rats (Dallegrave et al. 2007).

Similar kind of work was also done by Romano et al. to evaluate the perturbing effects caused by glyphosate on the reproductive system of the Wistar rats. Exposure of rats to the herbicide had significantly altered their progression of puberty. It had also reduced the testosterone production in male rats (Romano et al. 2010).

Swine Toxic effects of various components of glyphosate-surfactant herbicides on the cardiovascular system of higher mammals were evaluated by Lee et al. Five different groups of male piglets were infused with different concentrations of glyphosate along with the various components of glyphosate (used in commercial formulations). Results showed that the surfactants as well as the active component (isopropylamine salt of glyphosate) have caused detrimental effects on the cardiovascular system of the swine (Lee et al. 2009).

Humans Glyphosate is a water-soluble herbicide, so it gets accumulated in water and soil systems from where it enters the food chain. UK Food Standard Agency conducted residue testing of glyphosate in the samples of bread and found 0.2 mg/kg of glyphosate in 27 out of 109 samples. (Myers et al. 2016). US department of agriculture in 2011 divulges the presence of glyphosate residues in 90.3% of 300 soybean samples at the concentration of 1.9 ppm. Also, they detect the presence of residues of aminomethylphosphonic acid in 95.7% of soybean samples at concentration of 2.3 ppm (Osteen and Fernandez-Cornejo 2013). The presence of glyphosate and its metabolites in food is posing serious

threat to mankind and is disrupting the natural ecological balance. Various researchers have conducted toxicity tests to evaluate the harmful effects of the herbicide on humans.

Toxicity evaluation of glyphosate and its common formulation Roundup on the human placental JEG 3cells was carried by Richard et al. Results confirmed that glyphosate-based herbicides have perturbed the activity of enzyme aromatase (responsible for the synthesis of estrogen in females) and mRNA level present in the placenta of humans. Glyphosate also binds with the active sites of the enzyme and inhibits its activity. The toxic effects of glyphosate are facilitated by Roundup formulation. Roundup acts as a potent endocrine disruptor (Richard et al. 2005).

Cytotoxic and genotoxic properties of glyphosate and Roundup in the buccal epithelial cell line (TR146) of humans were evaluated by Koller et al. Workers were exposed to the herbicide via inhalation. Results confirmed that the use of Roundup damaged the epithelial cell membrane and also caused an impairment in mitochondrial functions. Significant increase in the nucleoplasmatic bridges nuclear aberrations and micronuclei indicates DNA damage. Moreover, an increased release of extracellular lactate dehydrogenase shows plasma damage in the cells. This study indicates that epithelial cells are more susceptible to the cytotoxic and DNA damaging by the use of these herbicides (Koller et al. 2012).

Mesnage et al. checked the toxic effects of glyphosate and its common adjuvant polyethoxylated tallowamine (POE-15) in nine glyphosate-based commercial formulations on the human cell line. Toxicity evaluation was performed on hepatic (Hep G2), embryonic (HEK 293) and placental (JEG 3) cell lines after 24-h exposure. Mitochondrial activities, membrane degradation and caspases 3/7 activities were taken as the criterion for toxicity evaluation. Results depicted that all the glyphosate formulations were more toxic than pure glyphosate. Adjuvant polyethoxylated tallowamine (POE-15) was found to be most toxic against human cells. The toxic effects of POE-15 were dose dependent and induce the necrosis of cells even after its first micellization process. However, glyphosate induced its endocrine disruption only after entering the cell. This study concluded that the addition of surfactants in the commercial formulations increases their toxicity (Mesnage et al. 2013).

Another research group evaluated the toxic effects of glyphosate-based herbicides, containing not only glyphosate but also different adjuvant on the 3T3-L1 cell line. Three different glyphosate formulations were taken to study their impact on 3T3-L1 fibroblast proliferation and differentiation. An abrupt increase in the cell number or cytosolic lipid accumulation was observed. The commercially available glyphosate formulations containing the adjuvant are more potent inhibitors of proliferation and cell differentiation of adipocytes of 3T3-L1 fibroblasts. This study demonstrates that not only polyethoxylated adjuvant but non-polyethoxylated adjuvant also contributes to cell toxicity. Thus, it was finally concluded that glyphosate-based herbicides disturbed the cell physiology and induces many cellular alterations (Martini et al. 2016).

Glyphosate's toxicity was also affirmed by another research group. According to them, glyphosate's toxicity is negligible when its residues were found in the food stuff like sugar, corn, soy and wheat. They intended that glyphosate interfered with cytochrome P450 (CYP) enzymes and interrupted the biosynthesis of aromatic amino acids by gut bacteria. It also impaired the serum sulfate transport in the blood. Disruption of CYP enzymes boosted the harmful effects of other food-borne chemical residues and environmental toxins which resulted in the damage of cellular systems all over the body. Eventually it makes the human body prone to many serious diseases like gastrointestinal disorders, obesity, heart disease, depression, autism, diabetes, infertility and cancer (Samsel and Seneff 2013).

Thongprakaisang et al. studied the toxic effects of technical grade glyphosate on the endocrine system of humans. Adverse effects of pure glyphosate were checked on estrogen receptors-mediated transcriptional activity and their expressions. They proposed that glyphosate exerts significant effects only in human hormone-dependent breast cancer T47D cells but does not affect hormone-independent breast cancer MDA-MB231 cells at 10^{-12} to 10^{-6} M in estrogen withdrawal condition. The considerable concentrations of glyphosate that persuade the commencement of estrogen response element transcription activity were 5–13 times more than in control (in T47D-KBluc cells). Also this activation was inhibited by an estrogen antagonist ICI 18278 which indicates that estrogenic activity of glyphosate was arbitrated by estrogen receptors. These findings suggested that the low and environmentally recommended concentrations of glyphosate can disrupt the hormonal system of humans (Thongprakaisang et al. 2013).

Another study by Gasnier et al. investigates the cytotoxicity, genotoxicity, anti-estrogenic and anti-androgenic effects of glyphosate-based formulations on human liver HepG2 cells. The cells were exposed to different concentrations of four different glyphosate formulations (Roundup Express, Bioforce, Grands Travaux and Grands Travaux plus) and pure glyphosate. Results suggested that all the parameters were disrupted with all the herbicidal formulations of glyphosate even after 24-h exposure. Concentrations above 0.5 mg/L of Grands Travaux (the most active formulation of glyphosate) caused human cell endocrine disruption on androgen receptor in MDA-MB453-kb2 cells. Concentration level above 2 mg/L inhibited transcriptional activities on estrogen receptors and hepatic cells HepG2. The concentration level above 10 mg/L caused severe cytotoxic effects

Table 3 Effect of glyphosate on vertebrates

Phylum	Species	Exposure time	Response	References
Fish	*Cyprinus carpio*	96 h	Increase in the alkaline phosphatase activity in liver, heart, increase in glutamic oxaloacetic transaminase activity in liver and kidneys, increase in glutamic pyruvic transaminase activity in the serum, epithelial hyperplasia and subepithelial edema, lifting and rupture of the respiratory epithelium and early signs of fibrosis	Neskovic et al. (1996)
	Salmo trutta	14 days	Increase in cell proliferation and cellular turnover	Webster and Santos (2015)
	Rhamdia quelen	96 h	Alterations in enzymatic activity, vacuolization, leukocyte infiltration, degradation of cytoplasm and melanomacrophage	Murussi et al. (2016)
	Cnesterodon decemmaculatus	90 days	Acetylcholinesterase activity in brain was decreased, liver glycogen content was reduced, hepatic glucose content was decreased, lactate level in liver and muscles was increased, and protein content in muscle was decreased	Menéndez-Helman et al. (2012)
	Jenynsia multidentata	7 and 28 days	Lifting of secondary lamellar epithelium, edema formation, hypertrophy of epithelial cells, hydropic degeneration in the liver, thickening of secondary lamellae in gills, focal necrosis and infiltration of leukocytes in liver	Hued et al. (2012)
	Pseudoplatystoma	96 h	Decrease in glucose level in plasma and increase in the glucose level of liver, lactate level increase in plasma and liver, decrease in protein and glycogen level of the plasma and muscles, decrease in cholesterol level, altered the enzymatic activity, ventilator frequency was increased and change in swimming behavior of fish	Sinhorin et al. (2014)
	Poecilia reticulate	24 h	Increase in the number of micronucleus and comets (increase in the number of damaged cells)	De Souza Filho et al. (2013)
	Pimephales promelas, Salmo gairdneri, Ictalurus punctatus, Lepomis macrochirus.	96 h	Toxicity effects depend upon the life cycle of the fish	Folmar et al. (1979)
	Cyprinus carpio	96 h	Repression of acetylcholinesterase activity, increase in thiobarbituric acid-reactive species and stimulation of lipid peroxidation	Cattaneo et al. (2011)

Table 3 (continued)

Phylum	Species	Exposure time	Response	References
Amphibians	*Leptodactylus latrans*	24 h	Affected swimming, oral abnormalities and edema	Bach et al.
	Leptodactylus latrans	48 h	Alterations in the hepatic tissue and erythrocyte nuclear abnormalities	Pérez-Iglesias et al. (2016)
	Rhinella arenarum	96 h	Breaking of DNA strands, lesions in peripheral blood cell	Soloneski et al. (2016)
	Rhinella arenarum	48 h	Neurotoxicity, oxidative stress and immunological depression	Lajmanovich et al. (2015)
	Lithobates catesbeianus	14 days	Significant reduction in glycogen and triglyceride level in all organs, increase in lipid peroxidation and cholesterol level in gills, decrease in total protein content in gills	Dornelles and Oliveira (2016)
	Rhinella arenarum	48 h	Decrease in the activity of important enzymes used in the catalyses of neurotransmitters	Lajmanovich et al. (2011)
	Rana clamitans, Rana sylvatica, Rana pipiens, Bufo americanus	96 h	Decreased snout-vent length, tail damage, gonadal abnormalities and delayed metamorphosis	Howe et al. (2004)
Reptiles	*Caiman latirostris*	2 months	Increase in the frequency of micronucleus, DNA damage with retarded growth	Gonzalez et al. (2013)
	Caiman latirostris	*In Ovo* exposure	DNA damage	Poletta et al. (2009)
	Caiman latirostris	2 months	Decrease in WBC count, increase in heterophils and total protein count	Latorre et al. (2013)
	Caiman latirostris	2 months	Decrease in heterophils and lymphocytes count	Siroski et al. (2016)
	Salvator merianae	*In Ovo* exposure	External structural abnormality, DNA damage	Schaumburg et al. (2016)
	Oligosoma polychrome	4 weeks	Increase in body metabolism and increase in body temperature	Carpenter et al. (2016)
Birds	*Agelazus phoeniceus, Cistothorus palustris, Xanthocephalus xanthocephalus*	–	Reduced the wetlands vegetation and affected bird population	Linz et al. (1996)
	Geothlypis trichas, Melospiza lincolnii, Empidonax alnorum	–	Affected versatility of birds and reduced their natural habitats and their breeding populations	Santillo et al. (1989)
	Anas platyrhynchos	15 days	Damage reproductive system of male bird and affected male genital organs	Oliveira et al. (2007)

Table 3 (continued)

Phylum	Species	Exposure time	Response	References
Mammals	Wistar Rats	2 months	Damaged mucosal epithelial cells, degeneration of hepatic cells, glomerular degeneration, tubular necrosis in kidneys, damaged spleen and pancreas	Tizhe et al. (2014a, b)
	Wistar Rats	21–23 days (during pregnancy), 21 days (during lactation)	Delay in vaginal canal opening in pregnant female rats, decrease in total sperm content in adult male rats, increase in the number of abnormal sperms and decrease in serum testosterone level	Dallegrave et al. (2007)
	Wistar Rats	–	Altered the progression of puberty in rats	Romano et al. (2010)
	Landrace piglets	24 and 48 h	Decreased cardiac index, increased pulmonary capillary wedge pressure, central venous pressure and mean pulmonary arterial pressure	
	Homo sapiens	18 h	Perturbed the activity of enzyme aromatase and mRNA level present in the placenta	Richard et al. (2005)
	Homo sapiens	20 min	Damaged epithelial cell membrane, impairment in mitochondrial functions, increase in nucleoplasmatic bridges, nuclear aberrations and micronuclei, increased release of extracellular lactate dehydrogenase	Koller et al. (2012)
	Homo sapiens	24 h	Negative dose-dependent effects on cellular respiration, necrosis of cells and endocrine disruption	Mesnage et al. (2013)
	Homo sapiens	24 h	Disturb cell physiology and induces cellular alterations	Martini et al. (2016)
	Homo sapiens	–	Interfered with cytochrome P450(CYP) enzymes, interrupted biosynthesis of aromatic amino acids and impaired the serum sulfate transport in the blood	Samsel and Seneff (2013)
	Homo sapiens	5 days	Disrupts hormonal system in humans	Thongprakaisang et al. (2013)
	Homo sapiens	24 h	Disruption of endocrine system, inhibited transcriptional activities on estrogen receptors, DNA damage	Gasnier et al. (2009)
	Homo sapiens	48 h	DNA damage with chromosomal aberrations	Manas et al. (2009)

610

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	Wistar Rats	21–23 days (during pregnancy), 21 days (during lactation)	Delay in vaginal canal opening in pregnant female rats, decrease in total sperm content in adult male rats, increase in the number of abnormal sperms and decrease in serum testosterone level	Dallegrave et al. (2007)
	Wistar Rats	–	Altered the progression of puberty in rats	Romano et al. (2010)
	Landrace piglets	24 and 48 h	Decreased cardiac index, increased pulmonary capillary wedge pressure, central venous pressure and mean pulmonary arterial pressure	
	Homo sapiens	18 h	Perturbed the activity of enzyme aromatase and mRNA level present in the placenta	Richard et al. (2005)
	Homo sapiens	20 min	Damaged epithelial cell membrane, impairment in mitochondrial functions, increase in nucleoplasmatic bridges, nuclear aberrations and micronuclei, increased release of extracellular lactate dehydrogenase	Koller et al. (2012)
	Homo sapiens	24 h	Negative dose-dependent effects on cellular respiration, necrosis of cells and endocrine disruption	Mesnage et al. (2013)
	Homo sapiens	24 h	Disturb cell physiology and induces cellular alterations	Martini et al. (2016)
	Homo sapiens	–	Interfered with cytochrome P450(CYP) enzymes, interrupted biosynthesis of aromatic amino acids and impaired the serum sulfate transport in the blood	Samsel and Seneff (2013)
	Homo sapiens	5 days	Disrupts hormonal system in humans	Thongprakaisang et al. (2013)
	Homo sapiens	24 h	Disruption of endocrine system, inhibited transcriptional activities on estrogen receptors, DNA damage	Gasnier et al. (2009)
	Homo sapiens	48 h	DNA damage with chromosomal aberrations	Manas et al. (2009)

with DNA damage at concentration of 5 mg/L (Gasnier et al. 2009).

Genotoxicity of glyphosate on humans was evaluated by Manas et al. by performing cytogenetic tests on Hep-2 cells. Four different concentrations (3, 4.50, 6 and 7.5 mM) of glyphosate were used for comet assay and chromosome aberrations test. A significant increase in DNA damage was observed at glyphosate concentration ranging from 3–7.5 mM. These observations showed the genotoxic nature of glyphosate in comet assay in Hep-2 cells (Manas et al. 2009).

Table 3 shows the toxic effects of glyphosate on various species of vertebrates.

Conclusion

Glyphosate is one of the most commonly used herbicide worldwide. Earlier it was thought that glyphosate is environment-friendly and does not cause any harm to nontarget organisms present in the ecosystem. But due to its overuse it has leached into ground water and soil systems where it is posing serious threats to the organisms found in aquatic and terrestrial systems. This review presents a complete toxicity evaluation of glyphosate and its common formulations on kingdom animalia and other lower group of organisms. Its noxious effects are not only bounded to unicellular organisms but also creating many distresses in multicellular organisms. It shows its negative effects from lower invertebrates to higher chordates. Overuse of glyphosate had seriously affected the earthworms by decreasing their rate of reproduction, loss of biomass, DNA damage and reduced surface casting activity. In aquatic systems, many lower invertebrates are also directly affected by the lethal nature of glyphosate. Apart from this, overuse of glyphosate in soil and its leaching in aquatic systems had reduced the egg-laying capacity and have hampered the hatching process in snails and sea urchins. Not only lower mammals but humans are also severely exaggerated by this herbicide. Roundup is found to be potent endocrine disruptor in human beings. It is causing serious damage to placental cells with the decrease in the activity of enzyme aromatase. It caused DNA damage, plasma damage and epithelial cell damage in humans. Surfactant polyethoxylated tallowamine showed harmful effects on hepatic, embryonic and placental cell lines.

Thus, we can conclude that the extreme use of herbicide glyphosate has caused toxic effects on nontarget organisms found in soil and water. It has affected almost all organisms of animal kingdom. This is a serious concern as it had affected the whole food chain and produced many unwanted changes in it. Glyphosate has reduced the availability of weeds which may be an important food source for many species. Thus, certain sustainable agricultural practices are needed to be adopted by farmers so as to maintain the interactions between biotic and abiotic components of ecosystems to get the ecological balance and to save the food webs.

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Chapter

Ecotoxicology of Glyphosate-Based Herbicides on Aquatic Environment

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Abstract

Glyphosate-based herbicides (GBHs) are chemicals developed to control unwanted plants such as weeds or algae. These chemicals act on EPSPS enzyme that blocks the production of tyrosine, phenylalanine, and tryptophan amino acids causing plant death. This biochemical pathway exists only in plant organisms. Despite the target use, GBHs have been related to toxic effects on nonplant organisms, such as invertebrates, fishes, amphibians, reptiles, birds, and mammals, including humans. This chapter is focused on ecotoxicological effects of GBHs on aquatic environment, showing a perspective of studies since this kind of product was developed until nowadays, an analysis of how many studies for each taxonomic group. Furthermore, we analyzed specifically the toxic effect of GBHs on each taxon, and finally, we discuss future perspectives and suggestions for a better regulation and application for this chemical.

Keywords: ecotoxicology, water quality, weed control, Roundup®, Monsanto

1. Introduction

Herbicides are chemical compounds used mostly to control weed (i.e., uncultivated) plants in agriculture and forestry and also for algae control [1, 2]. Herbicide formulations are designed to affect mainly plants, affecting specific plant biochemical pathways. However, it is common that this kind of pesticides affects nontarget organisms such animals, including aquatic organisms [3, 4].

The most used herbicide worldwide is glyphosate-based herbicide (GBH), such as Roundup® from Monsanto, and its usage has been increased [5] mainly due to the development of transgenic glyphosate-resistant crops [6]. Glyphosate (N-(phosphonomethyl) glycine (CAS no. 1071-83-6)) is a weak organic acid with a molecular weight of 169.09 M and has a half-life of 7–142 days in water and 76–240 in soil [6, 7]. Glyphosate has high solubility in water (10,000–15,700 mg L^{-1} at 25°C), and it readily dissolves and disperses in an aquatic environment.

1. Introduction

The use of agrochemicals is necessary to control pests and increase yields in order to produce adequate food for the global population, estimated at 6.8 billion in 2009 [1], and recently reported to have reached 7 billion [2]. Developing countries, where 1.02 billion people (15 %) are undernourished and 1.3 billion people (19 %) live on an inadequate diet [1], need an adequate food supply. However, the agricultural sector's annual application of over 140 billion kilograms of fertilizers and large amounts of pesticides creates massive sources of diffuse pollution of freshwater ecosystems [3]. In an attempt to increase food production, there is extensive use of herbicides without much regard to the consequences posed to the environment and humans. Glyphosate-based herbicides, which are extensively used in genetically modified glyphosate-resistant plants, are found all over the world [4] and have been reported to occur in various quantities in the aquatic ecosystem, wildlife and humans.

Globally, the presence of pesticides accumulation in both wildlife and humans is on ascendancy, with the health and normal functioning of the endocrine systems being at risk [5-7]. It is believed that the effects of these chemicals on normal functioning of the endocrine system are responsible for a number of developmental anomalies in a wide range of species, from invertebrates to higher mammals [8-11].

The aquatic environment is a receptacle of several undesirable contaminants, including agrochemicals. Therefore, contamination of the aquatic environment by pesticides has become a huge environmental concern worldwide [12]. Glyphosate and glyphosate-based herbicides are among the most widely used class of pesticides. Roundup®, is a major glyphosate-based herbicide used worldwide. Over the years, studies have suggested adverse effects of glyphosate and glyphosate-based compounds on terrestrial and aquatic environments, but recent publications are alluding to the possible effects of glyphosate on mammals, including humans, at different levels of biological organisations as well. In this chapter, the toxicology of glyphosate and glyphosate-based herbicides are explored. We reviewed the impacts of glyphosate and glyphosate-based herbicides on wildlife and humans using measured endpoint effects caused by genotoxicity, cytotoxicity and reproductive toxicity.

1.1. Pesticide as pollutants of freshwater ecosystems

Pesticides are mixtures of chemical substances designed to control, repel, mitigate, kill or regulate the growth of undesirable and nuisance biological organisms [13]. Pests include plant pathogens, weeds, nematodes, molluscs, insects, fish, birds, mammals and microorganisms such as bacteria and viruses. They compete with humans for food, transmit diseases and destroy crops as well as properties [13]. There are various ways of classifying pesticides, with the classification based on the type of pest they control being the most common. For example, insecticides, herbicides, fungicides, nematicides and rodenticides are used to control insects, weeds, fungi, nematodes and rodents, respectively. Furthermore, majority of pesticides are synthetic as they are formulated through industrial processes, while a few are biological as they are derived from natural sources. In addition, broad-spectrum pesticides are applied in

controlling a wide range of species but narrow-spectrum pesticides control a small group of pests [13].

Although pesticides are used in agriculture to maintain high production efficiency, they may be environmental hazards and pose risk particularly to non-targeted organisms, and generally to aquatic ecosystems [14, 15]. The potential of a pesticide's risk to an aquatic ecosystem is influenced by its properties, including half-life, mobility and solubility [13]. Microbial activity, drainage pattern, rainfall, treatment surface and application rate can also affect pesticidal activity on a local, regional or global scale [16, 17]. Pesticides get into aquatic systems through processes such as direct applications, surface runoffs, spray drifts, agricultural returns and groundwater intrusions [18]. Pesticides found in urban and agricultural settings in recent times have been implicated in the deaths of many aquatic biota [19].

1.2. Presence of herbicides in freshwater ecosystems

Weeds are unwanted vegetation, which are not planted intentionally, but inadvertently grow in unexpected places. They are usually controlled (i.e., killed or supressed) by the application of a specific herbicide type or class. Classification of herbicides may depend on the criteria used. The two most common criteria employed in herbicide classification are based on time of application and mode of action [20]. Table 1 shows herbicides classification based on time of application and mode of action. Herbicides, which are widely used to control weeds in forestry and agriculture, can reach the aquatic ecosystems by uncontrolled runoff, aerial drift or inadvertent overspray. In some cases, herbicides are directly sprayed at aquatic weeds (e.g. water hyacinth) found on surfaces of water bodies as a control measure. All these impact the aquatic biota.

Classification	Chemical family*	Examples
Time of herbicide application		
Pre-emergence: applied to the soil after the crop is planted	Dinitroaniline	Pendulum AquaCap Oryzalin (Surflan AS)
Post-emergence: applied to both crop and weeds after they have germinated and emerged from the soil	Benzoxazole	Acclaim® Extra
Mode of action		
Hormone inhibitors: These herbicides inhibit cell division and growth in the meristem regions (growing points) by mimicking IAA, the natural plant hormone. This interferes with cell wall plasticity and nucleic acid metabolism.	Phenoxycarboxylic acid	2,4-D 2,4-DB 2,4-DP MCPA MCPB
Cell division inhibitors: These herbicides bind to tubulin, the major microtubule protein, to form a herbicide–tubulin complex,	Carbamate	Chlorpropham Propham Carbetamide

Classification	Chemical family*	Examples
leading to a loss of microtubule structure and function. Herbicide-induced microtubule loss may cause cells to neither divide nor elongate, which may be observed as swelling of root tips.		
Photosynthesis inhibitors: These herbicides inhibit photosynthesis by preventing electron flow, CO_2 fixation and, ATP and NADPH2 production in the photosystem II complex in chloroplasts. Lack of ATP and NADPH2, as well as free radicals destroy cell membranes lead to eventual death of plant.	Triazine	Atrazine, Simazine, Caparol
Lipid synthesis inhibitors: These herbicides inhibit fatty acid and lipid biosynthesis. This causes reduction in cuticular wax development and eventual death of plant.	Thiocarbamate	Cycloate Dimepiperate Pebulate Thiobencarb Triallate
Cell metabolism inhibitors: These herbicides capture electrons from photosystem I, reduce them to form herbicide free radicals, which then destroy cell membranes.	Bipyridylium	Diquat Paraquat Gramoxone
EPSP Synthase Inhibitors: These herbicides inhibit EPSP synthase enzyme, which leads to the depletion of the aromatic amino acids tryptophan, tyrosine and phenylalanine.	Glycines	Glyphosate

Table 1. Examples of herbicide classification based on time of application and mode of action (* Note: there may be more than one chemical family for each category of herbicide)

The potential of some herbicides to control unwanted vegetation is inherent in their chemical nature, while others have additives to enhance their efficacy. These additives include carriers and adjuvants. In recent years, carriers and adjuvants have been implicated in adding to the toxicity of the active ingredients, and in some cases, have been even more toxic than the active ingredient alone [20]. Prior to the registration of herbicide products for use, not only does the herbicidal properties (Table 2) are assessed, but also the potential effects on humans, animals and environmental safety are assessed. The inherent toxicity of a herbicide, concentration to which an organism is exposed, and duration of exposure determine the extent to which the herbicide can adversely affect an aquatic organism [21]. Herbicides may reach aquatic ecosystems directly by an overhead spray of aquatic weeds, or indirectly through processes such as agricultural runoff, spray drift and leaching [13]. Potential problems associated with herbicide-use include injury to non-target vegetation, injury to crops, residue in soil or water, toxicity to non-target organisms, and concerns for human health and safety [20]. Herbicides

can influence the environmental water quality and ecosystem functioning by reducing species diversity, changing community structure, modifying food chains, altering patterns of energy flow and nutrient recycling, as well as reducing resilience of ecosystems [22].

Herbicidal property	Explanation
Chemical structure	The biologically active portion of a herbicide product is the *active ingredient*. It is the fundamental molecular composition and configuration of the herbicide. The physical and chemical properties of a herbicide can also determine the method of application and use.
Water solubility and polarity	Herbicides that are produced as salts dissolve quite well in water and are usually formulated to be applied in water, while non-polar herbicide sources are not. Water is the main substance used to disperse (spray) herbicides, and hence the water solubility of a herbicide influences the type of product that is formulated, how it is applied and the movement of the herbicide in the soil profile.
Volatility	Herbicides with a high vapour pressure volatilise easily, while those with a low vapour pressure are relatively non-volatile. The volatility of a herbicide can determine the mode of action and the herbicide's fate in the environment.
Formulations	Commercial herbicide products contain an active ingredient and "inert" ingredients. An "inert" ingredient could be a carrier that is used to dilute and disperse the herbicide (e.g. water, oil, certain types of clay, vermiculite, plant residues, starch polymers, certain dry fertilizers) or an adjuvant (e.g. activator, additive, dispersing agent, emulsifier, spreader, sticker, surfactant, thickener, wetting agent) that enhances the herbicide's performance, handling, or application.

Table 2. Herbicidal properties of herbicides that enhance their efficacy

2. Glyphosate and glyphosate-based herbicides

Glyphosate (N-(phosphonomethyl) glycine) (Figure 1) and glyphosate-based herbicides are the world's leading post-emergent, organophosphonate systemic, broad-spectrum and non-selective herbicides for the control of annual and perennial weeds [22, 23]. Worldwide, the number one glyphosate-based herbicide used is Roundup®. Other trade names of glyphosate-based herbicides include Roundup Ultra®, Roundup Pro®, Accord®, Honcho®, Pondmaster®, Protocol®, Rascal®, Expedite®, Ranger®, Bronco®, Campain®, Landmaster®, Fallow Master® and Aquamaster® manufactured by Monsanto; Glyphomax®, Glypro® and Rodeo® manufactured by Dow Agrosciences; Glyphosate herbicide manufactured by Du Pont; Silhouette® manufactured by Cenex/Land O'Lakes; Rattler® manufactured by Helena; MirageR® manufactured by Platte; JuryR® manufactured by Riverside/Terra; and Touchdown® manufactured by Zeneca [24-26].

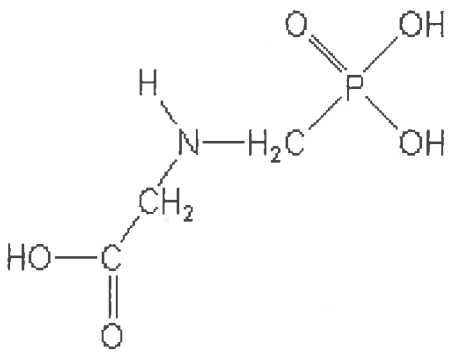

Figure 1. Molecular structure of N-(phosphonomethyl) glycine

Glyphosate has relatively low solubility in water (12 g/L at 25° C and 60 g/L at 100° C), but is insoluble in other solvents [27]. Therefore, commercial formulations of glyphosate are usually in the form of salt to ensure higher solubility yet maintaining the herbicidal properties of the parent compound [22]. Formulations of glyphosate in salt form include monoammonium salt, diammonium salt, isopropylamine salt, potassium salt, sodium salt, and trimethylsulfonium or trimesium salt. Of these, the isopropylamine, sodium, and monoammonium salt forms are commonly used in formulated herbicide products [28]. The isopropylamine salt is the most commonly used in commercial formulated products (e.g. Roundup®). The concentration of glyphosate is commonly expressed as mg a.i./L (active ingredient/Litre) or mg a.e./L (acid equivalents/Litre) [22]. Acid equivalent is the theoretical per cent yield of parent acid from a pesticide active ingredient, which has been formulated as a derivative (usually esters, salts or amines) [29].

2.1. Mode of action of glyphosate

As a systemic herbicide, glyphosate is readily translocated through the phloem to all parts of the plant. Glyphosate molecules are absorbed from the leaf surface into plant cells where they are symplastically translocated to the meristems of growing plants [22]. Glyphosate's phytotoxic symptoms usually start gradually, becoming visible within two to four days in most annual weeds, but may not occur until after seven days in most perennial weeds. Physical phytotoxic symptoms include progress from gradual wilting and chlorosis, to complete browning, total deterioration and finally, death [22]. The primary mode of action of glyphosate is confined to the shikimate pathway aromatic amino acid biosynthesis, a pathway that links primary and secondary metabolisms.

Shikimate (shikimic acid) is an important biochemical intermediary in plants and microorganisms, such as bacteria and fungi. It is a precursor for the aromatic amino acids phenylalanine, tryptophan and tyrosine. Other precursors of the shikimate pathway are indole, indole derivatives (e.g. indole acetic acid), tannins, flavonoids, lignin, many alkaloids, and other aromatic metabolites. The biosynthesis of these essential substances is promoted by enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), the target enzyme of glyphosate. This enzyme is one of the seven enzymes that catalyse a series of reactions, which begins with the

reaction between shikimate-3-phosphate (S3P) and phosphoenolpyruvate (PEP). The shikimate pathway accounts for about 35 % of the plant mass in dry weight and therefore any interference in the pathway is highly detrimental to the plant. Glyphosate inhibits the activity of EPSPS, preventing the production of chorismate – the last common precursor in the biosynthesis of numerous aromatic compounds in bacteria, fungi and plants. This causes a deficiency in the production of the essential substances needed by the organisms to survive and propagate [22, 30]. The pathway is absent in animals, which may account for the low toxicity of glyphosate to animals.

However, acute effects in animals, following intraperitoneal administration of high glyphosate doses, suggest altered mitochondrial activity, possibly due to uncoupling of oxidative phosphorylation during cellular respiration [27]. In summary, glyphosate ultimately interrupts various biochemical processes, including nucleic acid synthesis, protein synthesis, photosynthesis and respiration, which are essential life processes of living things.

2.2. Environmental fate of glyphosate

Glyphosate has a strong soil adsorption capacity, which limits its movement in the environment. The average half-life of glyphosate in soil is two months, but can range from weeks to years [24]. Glyphosate in freshwater ecosystems has an average half-life of two to ten weeks [24]. The rate of degradation in water is generally slower than in most soils because of fewer microorganisms in water than in soils [31]. When glyphosate undergoes degradation, it produces aminomethylphosphonic acid (AMPA) and carbon dioxide [32], both of which reduce pH when dissolved in water. However, pH is known to affect the stability of glyphosate in water. For instance, glyphosate did not undergo hydrolysis in buffered solution with a pH of 3, 6 or 9 at 35° C, while insignificant photodegradation has been recorded under natural light in pH 5, 7 and 9 buffered solutions [28]. In freshwater ecosystems, glyphosate dissipates through degradation, dilution, and adsorption on organic substances, inorganic clays and the sediment (the major sink for glyphosate in water bodies) [24, 31]. With its long half-life and its ability to cause death of organisms in aquatic ecosystems, it is recommended that glyphosate should be used as an aquatic herbicide to treat only one-third to half a water body at any one time [24].

2.3. Toxicology of glyphosate and its effects on aquatic organisms

In recent years, the exposure of non-target aquatic organisms to glyphosate-based herbicides has aroused great concern globally because of high water solubility and the extensive use of glyphosate-based herbicides [25]. In this regard, polyoxyethylene amine (POEA), a surfactant, has been implicated as being the main cause of the relatively high toxicity of Roundup® to several freshwater invertebrates and fishes [25, 33]. Technical grade glyphosate is slightly to very slightly toxic, with reported LC50 values of greater than 55 mg/L and a 21 d NOEC (no observed effect concentration) value of 100 mg/L [25, 33].

Conversely, formulations of glyphosate are moderately to very slightly toxic with 2 d EC50 values of 5.3-5600 mg/L and 21 d MATC values of 1.4-4.9 mg/L reported [27]. The LC50 values

also determine which glyphosate formulation can be applied in aquatic ecosystems. It should be noted that high LC50 value of a chemical substance to an organism implies low toxicity of that particular chemical substance to that particular organism, and the reverse is also true. For instance, Rodeo® has relatively high LC50s (>900 mg/L) for aquatic species and is permitted for use in aquatic ecosystems, while Touchdown 4-LC® and Bronco® have low LC50 values for aquatic species (<13 mg/L), and are not registered for aquatic use [24]. Similarly, Roundup® is not registered for use in aquatic ecosystems in the United States because its 96 h LC50 value for *Daphnia* is 25.5 mg/L, while that of glyphosate alone is 962 mg/L [24].

In recent years, glyphosate has been found in surface waters long after it has been used to control aquatic weeds, although it is generally regarded as having a low potential for contaminating surface waters [13, 34]. In fact, its mode of action was designed to affect only plants [30], but various studies in recent years have reported adverse impact on non-target animals [25, 33, 35].

2.4. Wildlife toxicology of glyphosate and glyphosate-based herbicides

Wildlife ecology is the application of ecological principles to the study of wildlife species. The term wildlife, however, lacks a universally accepted definition, and its common use changed during the 1900s in association with development of the profession of wildlife management. Historically, wildlife management focused on hunted or harvested birds and mammals that were collectively referred to as game species. However, current description of wildlife ecology includes different levels of biological organisation as well as individual organisms within a population and their interactions with the environment [36]. The effects of environmental contaminants on the health and persistence of wildlife populations have been a concern of environmentalists for many decades [36]. This led to increased interest in the study of exposure of wildlife to environmental contaminants, and hence wildlife toxicology.

A growing collection of wildlife toxicological studies examining diverse wildlife species demonstrates that exposure to environmental contaminants over the years is the cause of increasing disappearance of certain species. Such a threat to global biodiversity usually starts with developments of abnormalities in contaminated organisms. These may include the disruption of genetic material, cell integrity and major but subtle birth defects in individual species. This is against the belief, held for a long time since introduction of glyphosate as a commercial herbicide in the 1970s, that glyphosate is non-toxic to wildlife and humans. In fact, US EPA has classified glyphosate as a "Group E carcinogen", implying it is "non-carcinogenicity for humans" [37]. The main reason for this has been attributed to the fact that the herbicidal activity of glyphosate targets specifically inhibition of the shikimate pathway, which is only present in plants and microorganisms, but conspicuously absent in animals and humans [38]. However, recent studies seem to suggest that glyphosate has genotoxicity, cytotoxicity and reproductive toxicity in wildlife and humans [39-40].

Genotoxicity is a term used to describe the destructive effect by toxic agents referred to as genotoxins on a cell's genetic material (DNA, RNA). Genotoxins include both radiation and chemical genotoxins. There are three primary effects that genotoxins can have on organisms by affecting their genetic information depending on the type of genotoxin. These include

cancer-causing agents (carcinogens), mutation-causing agents (mutagens) or birth defect-causing agents (teratogens) [41]. Conversely, cytotoxicity is the term used to describe destructive effect of cells by agents referred to as cytotoxins. Cytotoxic cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis; they can stop growing and dividing; or they can activate a genetic program of controlled cell death, also referred to as apoptosis [41]. Reproductive toxicity refers to the toxic effects of a substance on the reproductive ability of an organism and the development of its offspring. The Globally Harmonized System (GHS) defines reproductive toxicity as adverse effects of chemical substances on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring [42]. Developmental toxicity refers to adverse effects induced during pregnancy, or as a result of parental exposure to a chemical substance manifested at any point in the life span of the organism [42]. Thus, reproductive toxicity may be grouped into two main categories: adverse effects on reproductive ability or capacity, and adverse effects on development of the offspring. Developmental toxicity and reproductive toxicity tests are usually performed with female and male animals, embryonic stem cells or whole embryos. In the following sections, we review a few of the many reported studies on adverse effects caused by glyphosate and glyphosate-based herbicides as a result of genotoxicity, cytotoxicity and reproductive toxicity in wildlife as well as in humans.

3. Toxicity of glyphosate and glyphosate-based herbicides to wildlife

It has been argued that the seasonality of crop cultivation and subsequent seasonal application of herbicides means that their presence in aquatic ecosystems is typically periodic. More so, episodic exposures are often for a short period, which is followed by long periods of non-application that has the capacity to "water down" the exposure effects. Therefore, the assessment of genotoxic endpoints in fish after removal of the contamination source is essential to understanding the dynamics of herbicide genotoxicity and risk posed by these agrochemicals. In order to test this assertion, Guilherme et al. [12] investigated the genotoxic potential of Roundup® to European eel (*Anguilla anguilla*) [12]. *A. anguilla* was exposed to 116 μg/L Roundup® for 3 days, and allowed to recover for 1, 7 and 14 days after exposure has ceased. A comet assay was applied to identify DNA damage in blood cells during both exposure and post-exposure periods. Guilherme et al. [12] reported that Roundup® has genotoxic potential and exhibited oxidative DNA damage (pyrimidine bases oxidation), although a recovery was evident when considering non-specific DNA damage on day 14 post exposure. Roundup® was able to induce a late oxidative DNA damage (day 14). In the report, Guilherme et al. suggested that blood cells of *A. anguilla* exposed to Roundup® appeared to be more successful in repairing damage with a non-specific cause than that associated with base oxidation [12]. Overall, the findings of this study reinforce the hypothesis that Roundup® as an agrochemical may cause long-lasting damage to fish due to genotoxicity.

In another study, Vera-Candioti et al. evaluated the genotoxicity and cytotoxicity of glyphosate-based formulations Panzer and Credit® after exposure to *Cnesterodon decemmaculatus* (Jenyns, 1842) (Pisces, Poeciliidae) under laboratory conditions [43]. They used micronuclei

induction (MN) and alterations in the erythrocytes to erythroblasts ratio for genotoxicity and cytotoxicity as endpoints, respectively. They reported that both 3.9 and 7.8 mg/L of Panzer increased MN frequency at 48 and 96 h of treatment. Similarly, *C. decemmaculatus* exposed to tested concentration of Credit® had increased frequency of MN over control group after 96 h, but not after 48 h. However, they reported that both Panzer and Credit® treatment did not cause cytotoxicity in fish, regardless of the concentration and sampling time. In their conclusion, Vera-Candioti et al. suggested that Panzer and Credit® should be considered as glyphosate-based commercial formulations with genotoxic but not cytotoxic effect properties [43].

Low levels of glyphosate-based herbicide have also been reported to cause adverse effects on reproduction (fecundity) and clutch size of aquatic invertebrates. In a study, Cuhra et al. reported that *Daphnia magna* exposed to 0.45 mg/L Roundup® significantly decreased fecundity compared to the control group [44]. They also reported that animals exposed to 1.35 mg/L Roundup® reached reproductive age, but almost all eggs and developing embryos were aborted and did not hatch, whereas animals exposed to 4.05 mg/L Roundup® died before reaching maturation. However, fecundity in *Daphnia magna* exposed to Roundup® concentrations of 0.05 and 0.15 mg/L was not significantly affected. The abortion rates for animals exposed to Roundup® concentrations of 0.05, 0.15 and 0.45 mg/L were not significantly different from those of the control group. On the other hand, the abortion rates for animals exposed to 1.35 mg/L of Roundup® were significantly higher than control group, reaching nearly 100 %. Cuhra et al. also reported that the size of first clutch (brood) juveniles born from groups exposed to 0.05, 0.15 and 0.45 mg/L Roundup® were not significantly different from the control group [44]. However the size of second clutch juveniles from animals exposed to 0.05, 0.15 and 0.45 mg/L Roundup® were significantly smaller than those of the control group. The authors contextualized their findings by comparing them to the general US EPA environmental guideline limit for glyphosate and the California State's specific EPA environmental guideline limit for glyphosate, which were 0.7 and 1.0 mg/L, respectively. These limits for glyphosate in surface waters are between the 0.45 and 1.35 mg/L concentrations Cuhra et al. used in their study [44]. They concluded that since *D. magna* showed complete reproductive failure and aborted all eggs in early-to-late stages of embryonic development when exposed to 1.35 mg/L suggests that the US EPA and the California State's environmental guidelines may not be sufficiently restrictive to ensure viable populations of *D. magna* and other aquatic invertebrates.

Studies with rats seemed to suggest that the respiratory and hepatic systems as well as reproductive functions including sperm production or libido, and even foetal development can be altered by exposure to Roundup®. This may be attributed to the fact that xenobiotics in the aquatic ecosystem may lead to endocrine disruption at a reproductive and more specifically testicular level in young as well as adult mammals [45]. For example, different forms of testicular dysgenesis (abnormal development and growth of the testicles) have been linked to xenobiotics in aquatic ecosystems. These include decrease in sperm quantity and quality, increase in congenital malformations such as cryptorchidism (the absence of one or both testes from the scrotum) and hypospadias (abnormally placed urinary meatus), and preoccupying increase of testicular cancer incidences [46-49].

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In a recent study, Prasad et al [50] investigated the genotoxic effects of glyphosate in the cells of Swiss Albino mice by measuring chromosomal aberrations (CAs) and micronuclei (MN) in bone marrow cells after exposure for 24, 48 and 72 h. Glyphosate treatment group mice were exposed by giving them a single dose of glyphosate intraperitoneally (i.p) at a concentration of 25 and 50 mg/kg b.wt. Simultaneously, positive control group mice were injected i.p. benzo(a)pyrene (100 mg/kg b.wt, once only), while control (vehicle) group mice were injected i.p. dimethyl sulfoxide (0.2 mL). Mice from all the groups were sacrificed at sampling times of 24, 48, and 72 h and their bone marrow analysed for cytogenetic and chromosomal damage. They reported that CAs and MN induction increased significantly in glyphosate treatment groups at both given dosages and time compared with the vehicle control group ($P < 0.05$). They also reported that glyphosate caused cytotoxic effects in the mice by significant decrease in mitotic index (MI). Based on their findings, Prasad et al [50] concluded that glyphosate is clastogenic and cytotoxic to mouse bone marrow.

In another study by Clair et al., mature rat fresh testicular cells were exposed to glyphosate and its formulation, Roundup®, from 1 to 10000 ppm [51]. This is the concentration range reported in some human urine and the environment, as well as in agricultural application levels. They found that Leydig cells got damaged from 1 to 48 h of Roundup® exposure, while other cells where damaged within 24-48 h; all mainly caused by necrosis. By contrast, glyphosate alone was toxic on Sertoli cells and later induced apoptosis at higher doses in germ cells and in Sertoli/germ cells co-cultures. At lower concentrations (i.e. 1 ppm), Roundup® and glyphosate were found to impact the endocrine system as they caused 35% decrease in testosterone, but only a high contamination appears to induce an acute rat testicular toxicity.

The effects of glyphosate on wildlife is not all that gloomy. At least, one study has reported this. Acacia et al. [52] studied the effects of glyphosate (as an active ingredient) and Roundup® (as a formulation) on oyster gametes and embryos to find a possible link between genotoxicity and reproduction/developmental impairment. They wanted to explore the impact of chemical genotoxicity on population dynamics of oysters since glyphosate is frequently found in oyster production areas, among other herbicides. Considering that oyster's gametes and embryos are in direct contact with the surrounding waters because its mode of reproduction is external, the presence of these agrochemicals does not only pose risk to oysters but also other aquatic organisms. In their study, Akcha et al. [52] exposed oyster spermatozoa and embryos to 0.5; 1.0; 1.5; 2.5; 5.0 µg active substance/L of both glyphosate and Roundup®. They reported that glyphosate and Roundup® had no effect on the oyster development at the concentrations tested. Their spermiotoxicity study also showed neither glyphosate nor Roundup® to be cytotoxic for oyster spermatozoa. It should be noted that although these findings by Akcha et al. [52] showed no negative effect on sperm function, the possible impact on fertilization rate and the consequences of the transmission of damaged DNA for oyster development and physiological performances was not investigated. More importantly, Akcha et al. [52] findings suggest that proper monitoring of usage of agrochemicals has the potential of minimising their toxic effects in the environment.

4. Toxicity of glyphosate and glyphosate-based herbicides to humans

Since the commercial introduction of glyphosate as a herbicide, its health effects have been studied intensely with the general conclusion that it is safe for humans [27, 42, 53]. However, recent published studies indicate that occupational exposure of humans to the herbicide is associated with increased cancer risks [54-55]. Also during the course of production of the herbicide, humans may come into dermal and/or inhalative contact with it. Although the shikimic pathway through which glyphosate disrupts plants biochemical activities is not found in animals, various studies seem to suggest the agrochemical may affect other pathways. The mitochondria and cytochrome P450 (CYP) pathways are thought to include possible candidate sites of action in animals, although there is no solid evidence to support such claims.

Mesnage et al. [56] investigated potential toxicity of 9 glyphosate-based formulations as well as technical grade glyphosate and polyethoxylated tallowamine POE-15 (the major adjuvant used in glyphosate-based formulations) to human cells, including hepatic (HepG2), embryonic (HEK293) and placental (JEG3) cell lines, after 24 h exposures [56]. They measured mitochondrial activities, membrane degradations and caspases 3/7 activities as endpoints. The authors reported that all formulations were more toxic than glyphosate, but POE-15 was found to be the most toxic against human cells, even if others were not excluded. The toxicity effect began with negative dose-dependent effects on cellular respiration and membrane integrity between 1 and 3 mg/L at environmental/occupational doses. They reported that POE-15 induced necrosis when its first micellisation process occurred, while glyphosate promoted endocrine disrupting effects after entering cells. The findings of Mesnage et al. challenged the establishment of guidance values such as the acceptable daily intake (ADI) of glyphosate since these are mostly based on a long term in vivo test of glyphosate alone [56]. The authors suggested that it is imperative to assess whole formulations of pesticides as mixtures with adjuvants that could change their toxicity in pesticide toxicity investigations.

Another study by Samsel and Seneff [57] asserted that glyphosate is minimally toxic to humans since residues are found in food stuff, including sugar, corn, soy and wheat. In their opinion, the disruption of cytochrome P450 (CYP) enzymes activities by glyphosate is an overlooked component of its toxicity to mammals. In a recent study, they reported that glyphosate interferences with cytochrome P450 (CYP) enzymes and acts synergistically to disrupt the biosynthesis of aromatic amino acids by gut bacteria, as well as impairment in serum sulphate transport. According to the authors, one of the many crucial functions of CYP enzymes is detoxification of xenobiotics. Therefore, by disrupting CYP enzymes activities, glyphosate enhances the damaging effects of other food borne chemical residues and environmental toxins. This adversely affects the body though the impact is subtle and manifests slowly over time as inflammation damages cellular systems throughout the body. Ultimately, these result in diseases and conditions such as gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's disease; mostly associated with a Western diet. Based on their study outcome, Samsel and Seneff described glyphosate as "textbook example" of exogenous semiotic entropy: the disruption of homeostasis by environmental toxins".

Further recent studies have also suggested glyphosate to be an endocrine disrupting chemical (EDC), which has the potential to cause adverse health effects in humans [58]. In a study to evaluate the EDC properties of glyphosate in humans, Thongprakaisang et al [58] investigated the effects of technical grade glyphosate on estrogen receptors (ERs) mediated transcriptional activity and their expressions [58]. They reported that the proliferative concentrations of glyphosate, which caused the activation of estrogen response element (ERE) transcription activity, were 5-13 fold more than the control in T47D-KBluc cells. However, the activation was inhibited by an estrogen antagonist, ICI 182780, which implied that the estrogenic activity of glyphosate was mediated via ERs. The findings of this investigation suggest that low and environmentally relevant concentration of glyphosate can disrupt the hormonal systems of humans. Furthermore, the effects demonstrate that glyphosate is or could act as a "xenoestrogen" and may be capable of inducing EREs in a manner slightly weaker but functionally similar to Estradiol (E2), the most potent human estrogen.

In another study to investigate the xenobiotic toxicity of glyphosate, Gasnier et al. exposed the human liver HepG2 cells to four different formulations and to glyphosate, and measured the cytotoxicity, genotoxicity, anti-estrogenic (on estrogen receptors (ERα) and (ERβ)) and anti-androgenic effects (on androgen receptor (AR)), as well as checked androgen to estrogen conversion by aromatase activity and mRNA [59]. They reported that all parameters were disrupted at sub-agricultural concentrations with all formulations within 24 h, with the effects more dependent on the formulation than on the glyphosate concentration. They also stated that concentration levels above 0.5 mg/L of the most active formulation (R400) caused a human cell endocrine disruption on the androgen receptor in MDA-MB453-kb2 cells, while concentration levels above 2 mg/L inhibited transcriptional activities on both ERs on the HepG2. The authors also reported that concentration levels above 10 mg/L disrupted aromatase transcription and activity, while cytotoxic effects started at concentration levels above 10 mg/L and DNA damages (genotoxic effects) at 5 mg/L.

Richard et al also investigated the effects of glyphosate and Roundup® on human placental JEG3 cells within 18 h with concentrations lower than those found with agricultural use [60]. They stated that both chemical substances were toxic to the human placental JEG3 cells and this effect increases with concentration and time but Roundup® was found to be more toxic than glyphosate. They also tested the effects of both chemical substances at lower environmentally nontoxic concentrations on aromatase, the enzyme responsible for estrogen synthesis. They reported that Roundup® disrupted aromatase activity and mRNA levels and interacted with the active site of the purified enzyme, but the effects of glyphosate were facilitated by adjuvants in microsomes or in cell culture. Based on their findings, Richard® et al suggested that glyphosate and Roundup® can induce endocrine and toxic effects in humans and other mammals.

In a separate study to ascertain whether glyphosate exposure may cause DNA damage and cancer in humans, Koller et al. investigated exposure of workers via inhalation to technical glyphosate and Roundup UltraMax, glyphosate-based herbicide [61]. They reported that the cytotoxic and genotoxic properties of glyphosate in the workers' buccal epithelial cell line (TR146) induced acute cytotoxic effects at concentration 40 mg/L after 20 min, which were due

to membrane damage and impairment of mitochondrial functions. Similarly, they stated that Roundup UltraMax induced release of extracellular lactate dehydrogenase at concentrations 80 mg/L, which indicates membrane damage. In their study, Koller et al. showed that both glyphosate and Roundup UltraMax induced DNA migration in single-cell gel electrophoresis assays at concentrations 20 mg/L [61]. The authors again showed that the frequencies of micronuclei and nuclear buds were elevated after 20 min exposure to 10-20 mg/L, as well as increase of nuclear aberrations that reflected DNA damage. However, nucleoplasmatic bridges were only enhanced by glyphosate at the highest dose (20 mg/L). The authors concluded that their findings suggest that inhalation of glyphosate and glyphosate-based herbicides by humans may cause DNA damage in exposed individuals since they found genotoxic effects after short exposure to concentrations that correspond to a 450-fold dilution of spraying used in agriculture [61].

5. Conclusion

In this chapter, we discussed the perceived "friendly" nature of glyphosate and glyphosate-based herbicides and their apparent non-toxicity to both wildlife and humans. This notion has been in existence ever since glyphosate was first introduced as a commercial herbicide in the 1970s largely because the herbicidal activity of glyphosate targets the inhibition of the shikimate pathway in particular, which is only present in plants and microorganisms. Thus glyphosate is classified by the US EPA as a Group E carcinogen, and therefore is non-carcinogenic to humans. However, current investigations involving glyphosate exposure to wildlife and humans show adverse effects resulting from genotoxicity, cytotoxicity and reproductive toxicity. These have been reviewed with examples in this chapter. Furthermore, we have demonstrated that the herbicide has an endocrine impact at very low environmentally relevant concentrations. This review of glyphosate herbicides needs to be taken seriously since the use of glyphosate-contaminated plant products as dietary supplements may pose a risk of cancer in humans because of their potential additive estrogenicity. In addition, the extensive use of glyphosate-based herbicides in genetically modified glyphosate-resistant plants grown for food and feed should be of grave concern since they can be sources of genotoxicity, cytotoxicity, and reproductive toxicity in wildlife and humans.

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Health and environmental impacts of glyphosate:

The implications of increased use of glyphosate in association with genetically modified crops

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This report was researched and written by David Buffin and Topsy Jewell of the Pesticide Action Network UK. Editing was by Pete Riley, Mary Taylor, Emily Diamand and Helen Barron

4

Environmental fate

When glyphosate is introduced into the environment a number of processes appear to determine its fate. The most important include:

- the formation of complexes in water with ions such as Ca2+, Mg2+
- sorption to sediment or suspended particles
- suspended particles in water and soil
- uptake and metabolism by plants
- biodegradation by micro-organisms.

A range of bacterial strains can degrade glyphosate using the compound as a source of phosphorus, carbon, or nitrogen. The major breakdown product or metabolite of glyphosate is aminomethylphosphonic acid (AMPA). Carbon dioxide is also a breakdown product[7].

4.1 Persistence in soil and water

The agrochemical company Zeneca claims that its product, Touchdown, a herbicide containing glyphosate trimesium, is "rapidly inactivated and broken down in the soil"[46]. Monsanto has measured the half life of glyphosate (the time required for half the amount to biodegrade or dissipate) and determined it to vary between three and 141 days.

In the field, long persistence of glyphosate has been observed in a number of studies. AMPA has been found to be even more persistent than glyphosate, with a half life in soil between 119 and 958 days[7]. In water, glyphosate has a long persistence in sediments. Records of glyphosate persistence include[47]:

- 249 days on Finnish agricultural soils.
- between 259 and 296 days on eight Finnish forestry sites.
- between one and three years on 11 Swedish forestry sites.
- 335 days on a Canadian forestry site.
- 360 days on three Canadian forestry sites.
- two Canadian studies found glyphosate persisted 12 to 60 days in pond water following direct application

- glyphosate residues in pond sediment were found 400 days after direct application with the formulation Accord
- glyphosate was found to persist for more than one year in studies of pond sediments in the US
- studies in Norway have detected glyphosate in surface and ground waters[48].

2 Mobility in soil

Monsanto claims glyphosate is essentially immobile in soil[9]. The belief that glyphosate readily and permanently binds to soil particles and remains in the upper few centimetres of soil has greatly increased its popularity and use. In reality, there is very little information available on the behaviour of glyphosate in soils[49]. The mechanism of sorption to soil is not fully understood, although it is believed that metal complexes with humic acid in soil may be the main binding mechanism[7].

Recent studies have cast important questions over the extent to which glyphosate is immobile in soil. One such study has shown that glyphosate can readily desorb from soil particles in some soil types and can be highly mobile in the soil environment (see below)[50]. Four soils, chosen to represent the most widespread soil types in the EU, were used in the study. The key findings included:

- Levels of adsorption of glyphosate varied in the different soils according to their composition. Least adsorption occurred in the soils containing lower levels of iron oxide. The clay mineral content was also found to be important. Soils containing higher levels of clay minerals adsorbed more glyphosate. However, desorption readily occurred in soil with a high clay mineral but low iron oxide content.
- Large parts of the fixed herbicide can be easily returned to the soil solution.
- The least adsorbing soils desorbed up to 80 per cent of the adsorbed herbicide and the high adsorbing soils released between 15 and 35 per cent of the glyphosate adsorbed.
- In soils that are unable to bind with glyphosate long enough for microbial degradation to take place, the herbicide can be extensively mobile in the soil environment.
- Desorbed glyphosate can leach to lower soil layers.
- Glyphosate can bond with water soluble humic substances found in soil solution. Humic substances are the soil components primarily responsible for the mobility of pesticides in soil. Glyphosate can be transported with humic substances to lower soil depths[50].

Other recent studies have found:

- Adsorption of glyphosate on clay minerals decreased in the presence of copper, due to the formation of glyphosate-copper complexes. The study concluded that in relation to glyphosate release and mobility in soil, it is necessary to take into account both the soil type and any element in the soil capable of forming complexes with glyphosate[51].
- A study of sandy soils in Western Australia found that adsorption of glyphosate and

AMPA increased strongly with iron and aluminium content of the soils, while soil organic matter competed for adsorption sites and inhibited adsorption[52].

In addition, there is some evidence that the presence of inorganic phosphate inhibits degradation of glyphosate by some bacteria. The WHO recommends that the effects of phosphate fertilisers on the binding of glyphosate to soils should be investigated[7].

1.3 Effects on soil micro- and macro-organisms

There is little available information on the biological effects of glyphosate in soil[7]. Experimental research suggests that some important beneficial soil bacteria and fungi, including nitrogen-fixing bacteria and fungi responsible for breaking down organic matter, are affected by glyphosate. Examples of these are included in Part 5. Some studies have shown the impacts of glyphosate treatment can last for several months. This suggests glyphosate can remain active and may be released from soil and taken up by organisms.

- As well as affecting nitrogen-fixing bacteria, glyphosate has been found to inhibit mycorrhizal fungi. Mycorrhizal fungi grow in a symbiotic relationship in or on the roots of higher plants, such as pine trees. They help plants absorb nutrients and help protect from cold or drought. The presence of the fungi is vital for the establishment and growth of seedling trees of a number of species[53].

Glyphosate has also been found to adversely affect earthworms:

- A study in New Zealand found that repeated biweekly applications of low rates of glyphosate (1/20 of typical rates) caused a reduction in growth, an increase in time to maturity and an increase in mortality of the most common earthworm found in agricultural soils[54].
- Other studies have found that glyphosate is toxic to earthworms[7]. Earthworms exposed to glyphosate-treated soils were soft, slack and lethargic at concentrations greater than 500 mg Roundup/kg dry soil weight. The response was dose related.

1.4 Effects on aquatic organisms

Glyphosate can contaminate surface water either directly as a result of aquatic weed control or indirectly when glyphosate bound to soil particles is washed into rivers or streams[7]. Glyphosate and commercially formulated products containing POEA surfactant are toxic to fish and to some aquatic invertebrates[7,12]. POEA is about 30 times more toxic to fish than glyphosate[55]. Studies have shown that the acute toxicity of glyphosate varies according to species and age of fish and under different environmental conditions, such as water hardness, pH and temperature[12].

Very little research has been performed on the effects of glyphosate on aquatic micro-organisms or invertebrates[56,57,58,59]. Similarly, few ecotoxicity studies have been performed with pond or river sediment and sediment-living organisms[7]. The WHO recommends the biological activity of sediment- and soil-bound glyphosate in the environment should be studied. The WHO also recommends that further toxicity studies of sediment-living organisms are needed[7].

The authors of one recent study were concerned that the lack of long-term exposure studies with sub-lethal levels of glyphosate has hindered the establishment of guidelines for levels of glyphosate for freshwater organisms[58]. The Australian National Registration Authority recently banned most formulations of glyphosate from use near water after tests found that surfactants used in most formulations are harmful to tadpoles. Monsanto*s new formulation Roundup Bioactive, which does not contain POEA, surfactants is excluded from the ban.

Recent studies that have tried to address the lack of information include:

- A study to determine the effects of sub-lethal glyphosate concentrations on carp. It found that sub-acute toxic effects included changes in some enzyme activity in serum, liver and kidneys and morphological changes in gills, liver and kidneys [59].
- A study in Louisiana, US, tested the effect of sub-lethal concentrations of glyphosate on an aquatic snail species, *Pseudosuccinea columella.* The study found that low levels of glyphosate adversely affect snail reproduction and development. It also found that, at different concentrations, glyphosate can stimulate growth and development and increase the number of eggs laid containing more than one embryo with the potential to increase the snail population. The snail is an intermediate host of sheep liver fluke and the study concluded that low levels of glyphosate could ultimately promote increased liver fluke infections in mammals[58].
- A study examined the DNA damage caused by five commercial pesticides, including Roundup, on bullfrog tadpoles. Significant increases in DNA damage were observed for two out of the three concentrations tested (both of which were well below the recommended application rates). There was a strong linear correlation between DNA damage and dose. The study concluded that Roundup is clastogenic (causes DNA damage) in tadpoles[60].
- A study to test the toxicity of 23 different pesticides on aquatic plant life found that diatoms and one cyanobacterium were sensitive to glyphosate. The study concluded that there are considerable differences in sensitivity among species and that the use of an uncertainty factor is needed to provide an acceptable margin of safety when evaluating the hazard of pesticides to the aquatic ecosystem[61].
- Another study found that glyphosate can potentially stimulate undesirable eutrophication effects if primary producers (diatoms, etc) use glyphosate as an alternative source of phosphorus. The study raised concerns that 'below detectable level* glyphosate induced eutrophication of waterways, could indirectly affect fish habitats, and have other aquatic resource management effects [57] . However, such an effect may not be significant in all cases as high levels of phosphorus can enter aquatic environments from other sources.

1.5 Effects on terrestrial organisms

Adverse effects of glyphosate and glyphosate-containing formulations have been recorded in a variety of terrestrial animal and plant species. Damage can result from direct toxicity effects, through damage to food supplies or habitat destruction.

Invertebrates

Studies have shown that glyphosate can have both a direct toxic effect and an indirect impact due to habitat change on forest-dwelling invertebrates:

- In the US, a three-year study found that herbivorous insects and ground invertebrates were significantly reduced up to three years after treatment with Roundup in a four-to-five-year-old clear-cut planted with spruce seedlings. The vegetation did not recover over the study period and the authors concluded that the effects on the forest organisms were mainly due to habitat change[62].
- A laboratory study found that Roundup exposure caused a decrease in the survival and a decrease in body weight of woodlice[63].

In the agricultural environment, the use of glyphosate has been found to adversely affect a number of species that are beneficial predators of crop pests. As early as the 1970s, the decrease in numbers of predatory arthropods and weed density following the use of herbicides was suggested as a cause for the increased frequency of cereal aphid outbreaks in treated fields[64]. Glyphosate and its commercial formulations have been found to have direct toxicity effects and indirect habitat impacts on both test and field populations of beneficial insects, mites and spiders:

- A study found that exposure to glyphosate killed more than 80 per cent of a test population of predatory beetle and 50 per cent of parasitoid wasp, lacewing, ladybird and predatory mite[65].
- A study of winter wheat fields in North Carolina, US, found that populations of large carabid beetles declined after treatment with a glyphosate formulation and did not recover for 28 days[66].
- A study of Roundup treatment of pasture hedgerows in the UK found a similar effect on carabid beetles. The study also found that Roundup treatment reduced the numbers of spiders, probably by killing the plants they preferred for web-spinning[67].
- A comparison of arthropod populations in sprayed and unsprayed headland plots in spring wheat fields in the UK, found that female carabid beetle species contained more eggs in unsprayed areas than in sprayed areas. The reduction in prey species in sprayed areas may have been affecting beetle fecundity and hence recruitment to beetle populations, with a corresponding decline in overall pest predation rates in the crop[68].

Birds and mammals

The toxicity of glyphosate to birds and mammals is generally low. Bird and mammal populations have been more severely affected by glyphosate-induced changes to their habitat and food sources. Studies on the effects of glyphosate on bird and mammal wildlife species have tended to focus on the effects of the use of glyphosate in forestry, particularly in North America, where glyphosate is used to remove plants that may compete for resources and light with conifer seedlings and trees. For example, in Canada, Monsanto*s Vision accounts for 81 per cent of all herbicide applications in forestry[69]. Deleterious impacts observed on small mammal populations in clear-cut forests sprayed with glyphosate are most probably due to habitat change and the decline in the availability of food (both plant and arthropod prey) and cover[62,70,71,72].

The following two studies show that small mammals exposed to glyphosate can be contaminated through their diet or direct contact and that exposure to glyphosate may affect behaviour.

C After aerial spraying of a forest in Oregon, US, with Roundup at a rate of 3.3 kg a.i./ha, concentrations of glyphosate in small mammals were of the same order of magnitude as the concentrations in litter and groundcover. The concentration of glyphosate in the internal organs of herbivorous small mammals decreased more slowly than in omnivorous and carnivorous small mammals. The highest concentration, 5 mg a.i./kg, was found in omnivorous deer mice immediately after spraying[73].

C Glyphosate and other environmental chemicals affected the taste and smell receptors in the gerbil. Glyphosate reduced responses to several taste solutions. Taste and smell are chemical responses that play a crucial role in food selection. Damage to taste and smell receptors can impair food intake, nutritional status and survival[74].

Another group of animals that has been studied for the effects of forest herbicides is songbirds. Changes to habitat diversity are also likely to be the cause of population-density reductions in song birds:

C A three-year study of four-to-five-year-old clear-cuts in Maine, US, planted with spruce seedlings and sprayed with glyphosate at a rate of 1.7 kg a.i./ha found that total bird densities decreased by 36 per cent. The most sensitive species were the insectivorous common yellowthroat, lincoln*s sparrows and alder flycatchers[75].

C A study in Nova Scotia found that the densities of two common species of birds, white-throated sparrow and common yellowthroat, declined for two years after clear-cuts were sprayed with glyphosate. Densities had returned to normal by the fourth year. However, it was observed that new species including warblers, vireos, and a hummingbird had colonised the unsprayed control plot. These species did not appear in the sprayed plots[76].

C In Norway, black grouse avoided clear-cuts for several years after spraying with

glyphosate. The authors recommended that glyphosate not be used near grouse courtship areas[77].

In the UK, the indirect effects of pesticides on farmland bird populations has been a subject of concern for the Royal Society for the Protection of Birds (RSPB). Cereal herbicides are associated with the decline of 11 species of farmland birds[78].

Plants

Glyphosate is also a threat to terrestrial non-target plants as a result of spray drift from target areas. Measurement of the effects of herbicide spray drift on plant communities is very difficult. This is especially true when the amounts received are sub-lethal and the long-term damage is likely to be subtle[79]. In the US, for example, sub-lethal doses of herbicides have been blamed for reducing winter hardiness and resistance to fungal diseases in trees[80]. Studies of the impact of spray drift include:

- A study of the effects of spray drift of a glyphosate formulation on British species commonly found in nature reserves. The plant species were exposed to spray drift at different distances, wind speeds and application rates (0.5 and 2.2 kg a.i./ha). Death and severe growth suppression occurred at a distance of 2-6 metres from the sprayer. Sub-lethal effects could be detected up to 20 metres away for one species, *Prunella vulgaris* (self heal). Some species were consistently more sensitive including *Digitalis purpurea* (foxglove), *Centaurea nigra* (hard head), *Prunella vulgaris* (self heal) and *Lychnis flos-cuculi* (ragged robin). Epinasty (more rapid growth of the upper side of an organ causing for example curling in a leaf) was the most frequent symptom of damage[81].
- A recent study looked at species typical to UK woodland margins, hedgerows and field margins. The plant communities were exposed to glyphosate and other herbicides each year for at least three years. The effects of sub-lethal doses were measured on species yield, flowering performance, seed production, seed variability and invasion of new species. All species showed some effects within an eight-metre zone. One important result of this study was the finding that individual species respond to spray drift in different ways depending on the structure of the plant community in which they are growing. This means that it is extremely difficult to extrapolate the results of toxicology tests on single species to predict an outcome in the field[79].
- A UK Forestry Commission study into the decline of hedgerow ash found that 19 per cent of hedgerow ash showed symptoms of dieback (measured according to the proportion of foliage lost). Trees in rural areas were more badly affected than urban trees. In rural areas, dieback was strongly associated with arable land (38 per cent of trees associated with arable land suffered dieback compared to 10 per cent for trees surrounded by grassland). The Forestry Commission believes that hormone and glyphosate herbicides commonly affect hedgerow trees and may in part be responsible for the dieback in ash. Other factors may include plough damage to the trees' root system and soil compaction by farm vehicles[82].

The introduction of crops genetically engineered to tolerate glyphosate poses an additional threat to plant wildlife. Some crops have wild relatives with which they can cross pollinate. There is therefore a risk of introducing engineered genetic material into the wild population. The ecological implications of this genetic pollution in wild populations is unpredictable and may be similar to the introduction of foreign species. Most introduced non-indigenous plants do not survive. But those that do can cause massive economic damage. If the modified gene enhances the survival of the wild species it may give it an advantage over other wild species and ultimately affect the structure of local plant communities[83]. A number of studies have demonstrated the potential for transfer of tolerant genes to wild species and these are discussed later under the section on 'Impacts of genetically modified glyphosate-tolerant crops'.

Glyphosate, Part 1: Toxicology.

Caroline Cox. Journal of Pesticide Reform, Volume 15, Number 3, Fall 1995. Northwest Coalition for Alternatives to Pesticides, Eugene, OR.

Glyphosate, Part 1: Toxicology

by Caroline Cox

Introduction

Glyphosate is a broad-spectrum herbicide widely used to kill unwanted plants both in agriculture and in nonagricultural landscapes. Estimated use in the U.S. is between 19 and 26 million pounds per year.

Most glyphosate-containing products are either made or used with a surfactant, chemicals that help glyphosate to penetrate plant cells.

Glyphosate-containing products are acutely toxic to animals, including humans. Symptoms include eye and skin irritation, cardiac depression, gastrointestinal pain, vomiting, and accumulation of excess fluid in the lungs. The surfactant used in a common glyphosate product (Roundup) is more acutely toxic than glyphosate itself; the combination of the two is yet more toxic.

In animal studies, feeding of glyphosate for three months caused reduced weight gain, diarrhea, and salivary gland lesions. Lifetime feeding of glyphosate caused excess growth and death of liver cells, cataracts and lens degeneration, and increases in the frequency of thyroid, pancreas, and liver tumors.

Glyphosate-containing products have caused genetic damage in human blood cells, fruit flies, and onion cells.

Glyphosate causes reduced sperm counts in male rats, a lengthened estrous cycle in female rats, and an increase in fetal loss together with a decrease in birth weights in their offspring.

It is striking that laboratory studies have identified adverse effects of glyphosate or glyphosate-containing products in *all* standard categories of toxicological testing.

Two serious cases of fraud have occurred in laboratories conducting toxicology and residue testing for glyphosate and glyphosate-containing products.

Advertised as herbicides that can "eradicate weeds and unwanted grasses effectively with a high level of environmental safety,"1 glyphosate-based herbicides can seem like a silver bullet to those dealing with unwanted vegetation. However, an independent, accurate evaluation of their health and environmental hazards can draw conclusions very different than those presented by these advertisements. The following summary of glyphosate's hazards is intended to serve that purpose. It will appear in two parts: Part 1 discusses the toxicology of glyphosate, its metabolites, and the other ingredients of glyphosate products and Part 2 will discuss human exposure to glyphosate and its ecological effects.

Glyphosate, N-(phosphonomethyl) glycine (Figure 1), is a post-emergent, systemic, and non-selective herbicide used to kill broad-leaved, grass, and sedge species.2 It has been registered as a broad spectrum herbicide in the U.S. since 1974 and is used to control weeds in a wide variety of agricultural, lawn and garden, aquatic, and forestry situations.3

Most glyphosate herbicides contain the isopropylamine salt of glyphosate. A related chemical, the sodium salt of glyphosate, acts as a growth regulator in sugar cane and peanuts and is marketed for that purpose.

The monoammonium salt of glyphosate is also marketed as an herbicide and growth regulator.4

Glyphosate products are manufactured by Monsanto Company worldwide. The herbicide is marketed under a variety of trade names: Roundup (including Roundup D-Pak, Roundup Lawn and Garden Concentrate, and Roundup Ready-to-Use) and Rodeo are the most common U.S. trade names.2 The sodium salt is sold as Quotamaster. The monoammonium salt is sold as Deploy Dry.2 Other brand names used for the isopropylamine salt are Accord,5 Vision, Ranger, and Sting.2

As an herbicidal compound, glyphosate is unusual in that essentially no structurally related compounds show any herbicidal activity.6

Use

Glyphosate is the eighth most commonly used herbicide in U.S. agriculture and the second most commonly used herbicide in nonagricultural situations. Estimated annual use according to the U.S. Environmental Protection Agency (EPA) is between 15 and 20 million pounds in agriculture and between 4 and 6 million pounds elsewhere.7 The largest agricultural uses are in the production of soybeans, hay and pasture, corn, and oranges.4

About 25 million applications per year are made in U.S. households; most of these are made on lawns or outdoor areas where a total vegetation kill is wanted.8

In California, where pesticide use reporting is more comprehensive than in other states, about 3.4 million pounds were used in 1992; about 25 percent of this was used along rights-of-way, while 15 percent was used on almonds and 10 percent was used on grapes.9

Mode of Action

The mode of action of glyphosate is "not known at this time,"4 according to EPA. However, "herbicidal action probably arises from the inhibition of the biosynthesis of aromatic amino acids."10 These amino acids (phenylalanine, tyrosine, and tryptophan) are used in the synthesis of proteins and are the essential for growth and survival of most plants. One particular enzyme important in aromatic amino acid synthesis, called 5-enolpyruvylshikimate-3- phosphate synthase, is inhibited by glyphosate.10 Glyphosate also "may inhibit or repress"4 two other enzymes, chlorismate mutase and prephrenate hydratase, involved in other steps of the synthesis of the same amino acids. These enzymes are all part of what is called the shikimic acid pathway, present in higher plants and microorganisms but not in animals.11

Two of the three aromatic amino acids (tryptophan and phenylalanine) are essential amino acids in the human diet because humans, like all higher animals, lack the shikimic acid pathway, cannot synthesize these amino acids, and rely on their foods to provide these compounds. Tyrosine is synthesized in animals through another pathway.12

Glyphosate can affect enzymes not connected with the shikimic acid pathway. In sugar cane, it reduces the activity of one of the enzymes involved in sugar metabolism, acid invertase. This reduction appears to be mediated by auxins, plant hormones.13

Glyphosate also affects enzyme systems found in animals and humans. In rats, injection into the abdomen decreases the activity of two detoxification enzymes, cytochrome P-450 and a monooxygenase, and decreases the intestinal activity of the enzyme aryl hydrocarbon hydroxylase (another detoxification enzyme).14

"Inert" Ingredients in Glyphosate-containing Products

Virtually every pesticide product contains ingredients other than what is called the "active" ingredient(s), those designed to provide killing action. Their purpose is to make the product easier to use or more

efficient. These ingredients are called "inert," although they are often not biologically, chemically, or toxicologically inert. In general, they are not identified on the label of the pesticide product.

In the case of glyphosate products, many "inerts" have been identified. Roundup contains a polyethoxylated tallowamine surfactant (usually abbreviated POEA), related organic acids of glyphosate, isopropylamine, and water. Both Rodeo and Accord contain glyphosate and water.15 (However, label instructions usually require adding a surfactant during use.15) See "Toxicology of 'Inert' Ingredients of Glyphosate- containing Products," p. 17, for basic information about these "inert" ingredients.

Many of the toxicology studies that will be summarized in this factsheet ~~have been~~ conducted using glyphosate, the active ingredient, alone. Some have been conducted with commercial products containing glyphosate and "inert" ingredients. When toxicology testing is not done with the product as it is actually used, it is impossible to accurately assess its hazards.

We will discuss both types of studies, and will identify insofar as is possible exactly what material was used to conduct each study.

Acute Toxicity to Laboratory Animals

Glyphosate's acute oral median lethal dose (the dose that causes death in 50 percent of a population of test animals; LD50) in rats is greater than 4,320 milligrams per kilogram (mg/kg) of body weight. This places the herbicide in Toxicity Category III (Caution).4 Its acute dermal toxicity (dermal LD50) in rabbits is greater than 2,000 mg/kg of body weight, also Toxicity Category III.4

If animals are given glyphosate in other ways, it is much more acutely toxic. When given intraperitoneally (the dose applied by injection into the abdomen), glyphosate is between 10 and 20 times more toxic to rats (with an LD50 between 192- 467 mg/kg)2,16 than it is when given orally. Intraperitoneal injection also caused fever, cessation of breathing, and convulsions.17 While this kind of exposure is not one that would be encountered under conditions of normal use, these studies indicate the kinds of effects glyphosate can potentially cause in mammals.

Commercial glyphosate-containing products are more acutely toxic than glyphosate alone. Two recent (1990 and 1991) studies compared the amount of Roundup required to cause death in rats with the amount of either glyphosate alone or POEA alone that would cause death. The studies found that in combination, the amount of glyphosate and POEA required to kill was about 1/3 of a lethal dose of either compound separately. The Roundup formulation tested was also more toxic than POEA alone.18,19

As with glyphosate alone, glyphosate-containing products are more toxic when administered other ways than orally. Inhalation of Roundup by rats caused "signs of toxicity in all test groups,"20 even at the lowest concentration tested. These signs included a dark nasal discharge, gasping, congested eyes, reduced activity, hair standing erect,21 and body weight loss following exposure.20 Lungs were red or blood-congested.21 The dose required to cause lung damage and mortality following pulmonary administration of Roundup Lawn and Garden Concentrate or Roundup-Ready-to-Use (the glyphosate product is directly forced into the trachea, the tube carrying air into the lungs) was only 1/10 the dose causing damage through oral administration.18

Effects on the Circulatory System: When dogs were given intravenous injections of glyphosate, POEA, or Roundup so that blood concentrations were approximately those found in humans who ingested glyphosate, a variety of circulatory effects were found. Glyphosate increased the ability of the heart muscle to contract. POEA reduced the output of the heart and the pressure in the arteries. Together (Roundup), the result was cardiac depression.22

Eye Irritation: Glyphosate is classified as a mild eye irritant by EPA, with effects lasting up to seven days4 although more serious effects were found by the World Health Organization. In two of the four studies they reviewed, glyphosate was "strongly irritating"2 to rabbits' eyes and a third test found it

"irritating."2 In tests of glyphosate- containing products, all eight products tested were irritating to rabbit eyes, and four of the products were "strongly" or "extremely" irritating.2

Skin Irritation: Glyphosate is classified as a slightly irritating to skin. Roundup is a "moderate skin irritant" and causes redness and swelling on both intact and abraded rabbit skin. Recovery can take more than two weeks.20

Acute Toxicity to Humans

The acute toxicity of glyphosate products to humans was first widely publicized by physicians in Japan who studied 56 cases of Roundup poisoning. Most of the cases were suicides or attempted suicides; nine cases were fatal. Symptoms of acute poisoning in humans included gastrointestinal pain, vomiting, excess fluid in the lungs, pneumonia, clouding of consciousness, and destruction of red blood cells.23 They calculated that the mean amount ingested in the fatal cases was slightly more than 200 milliliters (about 3/4 of a cup). They believed that POEA was the cause of Roundup's toxicity.23 More recent reviews of glyphosate poisoning incidents have found similar symptoms, as well as lung congestion or dysfunction,24-26, erosion of the gastrointestinal tract,24,26 abnormal electrocardiograms,26 massive gastrointestinal fluid loss,27 low blood pressure,23,26 and kidney damage or failure.24,25,27

Smaller amounts of Roundup also cause adverse effects. In general these include the skin or eye irritation documented in animal studies, as well as some of the symptoms seen in humans following ingestion. For example, rubbing of Roundup in an eye caused swelling of the eye and lid, rapid heartbeat, palpitations, and elevated blood pressure. Wiping the face with a hand that had contacted leaky Roundup spray equipment caused a swollen face and tingling of the skin. Accidental drenching with Roundup (horticultural strength) caused recurrent eczema of the hands and feet lasting two months.25

Different symptoms have been observed when a different type of exposure has occurred. In Great Britain, a study compared the effects of breathing dust from a flax milling operation that used flax treated with Roundup with the effects of dust from untreated flax. Treated flax dust caused a decrease in lung function and an increase in throat irritation, coughing, and breathlessness.28

Subchronic Toxicity

Experiments in which glyphosate was fed to laboratory animals for 13 weeks showed a variety of effects. In experiments conducted by the National Toxicology Program (NTP), microscopic salivary gland lesions were found in all doses tested in rats (200 - 3400 mg/kg per day) and in all but the lowest dose tested in mice (1,000-12,000 mg/kg per day). Both the parotid and submandibular salivary glands were affected in rats; in mice the lesions were confined to the parotid gland. Based on further experiments, NTP concluded the lesions were mediated by the adrenal hormone adrenalin.29

The NTP study also found evidence of effects on the liver: increases in bile acids as well as two liver enzymes were found in both males and females. Other effects found in this study were reduced weight gain in male and female rats and mice; diarrhea in male and female rats; and changes in the relative weights of kidney, liver and thymus in male rats and mice.29

Other subchronic laboratory tests found decreased weight gains (using doses of 2500 mg/kg per day)30 along with an increase in the weights of brain, hearts, kidney, and livers in mice.2 In rats, blood levels of potassium and phosphorus increased at all doses tested (60-1600 mg/kg/day) in both sexes. There was also an increase in pancreatic lesions in males.4

As in acute toxicity tests, glyphosate-containing products are more toxic than glyphosate alone in subchronic tests. In a 7 day study with calves, 790 mg/kg of Roundup caused labored breathing, pneumonia, and death of 1/3 of the animals tested. At lower doses decreased food intake and diarrhea were observed.2

Chronic Toxicity

Glyphosate is also toxic in long-term studies. The following effects were found in lifetime glyphosate feeding studies using mice: decreased body weight, excessive growth of particular liver cells, death of the same liver cells, and chronic inflammation of the kidney. Effects were significant only in males and at the highest dose tested (about 4800 mg/kg of body weight per day). In females, excessive growth of some kidney cells occurred.31 At a lower dose (814 mg/kg of body weight per day) excessive cell division in the urinary bladder occurred.2

Lifetime feeding studies with rats found the following effects: decreased body weight in females; an increased incidence of cataracts and lens degeneration in males; and increased liver weight in males. These effects were significant at the highest dose tested (900-1200 mg/kg of body weight per day).4 At a lower dose (400 mg/kg of body weight per day) inflammation of the stomach's mucous membrane occurred in both sexes.2

Carcinogenicity

The potential of glyphosate to cause cancer has been a controversial subject since the first lifetime feeding studies were analyzed in the early 1980s. The first study (1979-1981) found an increase in testicular interstitial tumors in male rats at the highest dose tested (30 mg/kg of body weight per day).32 as well as an increase in the frequency of a thyroid cancer in females.33 The second study (completed in 1983) found dose-related increases in the frequency of a rare kidney tumor in male mice.34 The most recent study (1988-1990) found an increase in the number of pancreas and liver tumors in male rats together with an increase of the same thyroid cancer found in the 1983 study in females.35

All of these increases in tumor incidence are "not considered compound-related"35 according to EPA. In each case, different reasons are given for this conclusion. For the testicular tumors, EPA accepted the interpretation of an industry pathologist who said that the incidence in treated groups (12 percent) was similar to those observed in other control (not glyphosate-fed) rat feeding studies (4.5 percent).36 For the thyroid cancer, EPA stated that it was not possible to consistently distinguish between cancers and tumors of this type, so that the incidences of the two should be considered together. The combined data are not statistically significant.33 For the kidney tumors, the registrants reexamined slides of kidney tissue, finding an additional tumor in untreated mice so that statistical significance was lost. This was despite a memo from EPA's pathologist stating that the lesion in question was not really a tumor.34 For the pancreatic tumors, EPA stated that there was no dose-related trend and no progression to malignancy. For the liver tumors and the thyroid tumors, EPA stated that pairwise comparisons between treated and untreated animals were not statistically significant and there was no progression to malignancy.35

EPA concluded that glyphosate should be classified as Group E, "evidence of non-carcinogenicity for humans."35 They added that this classification "is based on the available evidence at the time of evaluation and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances." 35 From a public health perspective, the results of the laboratory tests leave many questions unanswered. An EPA statistician wrote in a memo concerning one of the carcinogenicity studies, "Viewpoint is a key issue. Our viewpoint is one of protecting the public health when we see suspicious data."36 Unfortunately, EPA has not taken that conservative viewpoint in its assessment of glyphosate's cancer-causing potential.

There are no studies available to NCAP evaluating the carcinogenicity of Roundup or other glyphosate-containing products. Without such tests, the carcinogenicity of glyphosate-containing products is unknown.

Mutagenicity

Laboratory studies of a variety of organisms have shown that glyphosate-containing products cause genetic damage:

* In fruit flies, Roundup and Pondmaster (an aquatic herbicide consisting of glyphosate and a trade secret surfactant)37 both increased the frequency of sex-linked, recessive lethal mutations. (These are mutations that are usually visible only in males because two damaged genes are required in order to be expressed in females.) In this study, the frequency of lethal mutations was between 3 and 6 times higher in fruit flies that had been exposed to glyphosate products during their larval development than in unexposed flies.38

* A laboratory study of human lymphocytes (one type of white blood cell) showed an increase in the frequency of sister chromatid exchanges following exposure to high doses of Roundup.39 (Sister chromatid exchanges are exchanges of genetic material during cell division between members of a chromosome pair. They result from point mutations.)

* In Salmonella bacteria, Roundup was weakly mutagenic at high concentrations. In onion root cells, Roundup caused an increase in chromosome aberrations.40

Glyphosate alone has rarely caused genetic damage in laboratory tests. None of the mutagenicity studies required for registration of glyphosate have shown it to be mutagenic. Tests included studies of mutations in hamster ovary cells, bacteria, and mouse bone marrow cells.4 Glyphosate was also not mutagenic in other studies of rats, mice,2 and onion cells40 but caused chromosome stickiness and fragmentation in water hyacinth root cells.41

Reproductive Effects

Laboratory studies have demonstrated a number of effects of glyphosate on reproduction, including effects on mothers, fathers, and offspring.

In rat feeding studiess, glyphosate reduced sperm counts (at the two highest doses tested) and lengthened the estrous cycle, how often a female comes into heat (at the highest dose tested).29 Other effects on mother rats in laboratory tests include soft stools, diarrhea, breathing rattles, red nasal discharge, reduced activity, growth retardation, decreased body weights, and increased mortality.2 Effects on offspring included an increase in fetal loss, a decrease in the number of embryos successfully implanted into the uterus, a decrease in the number of viable fetuses, a slight decrease in litter size, a decrease in fetal and pup weights, and an increase in problems with breast bone formation.2 Effects were observed at the highest doses tested (1500 and 3500 mg/kg of body weight per day).2

In a study of rabbits using doses that were lower than those used in the rat studies above, glyphosate caused diarrhea, nasal discharge, and death in mothers.2 The only effect on offspring was a decrease in fetal weight in all treated groups.42

A study in which glyphosate was fed to rats for three generations after which the offspring were examined for birth defects found kidney damage at a relatively low dose (30 mg/kg of body weight). However, a second study (only two generations long) did not find similar effects, and EPA called the damage in the first study "spurious."4 From a public health perspective, however, a new three generation study is crucial.

Toxicology of Glyphosate's Major Metabolite

In general, studies of the breakdown of glyphosate find only one metabolite, aminomethylphosphonic acid (AMPA).2 (See Figure 5.) Although AMPA has low acute toxicity (its LD50 is 8,300 mg/kg of body weight in rats)20 and is only slightly irritating to eyes,43 it causes a variety of toxicological problems. In subchronic tests on rats, AMPA caused decreased weight gain in males; an increase in the acidity of urine in both males and females; an increase in the activity of an enzyme, lactic dehydrogenase, in both sexes; a decrease in liver weights in males at all doses tested; and excessive cell division in the lining of the urinary bladder and in part of the kidney in both sexes.20 AMPA is much more persistent than glyphosate; studies in eight states found that the half-life in soil (the time required for half of the original concentration of a compound to break down or dissipate) were between 119 and 958 days.2

Quality of Toxicology Testing

Tests done on glyphosate to meet registration requirements have been associated with fraudulent practices.

Laboratory fraud first made headlines in 1983 when EPA publicly announced that a 1976 audit had discovered "serious deficiencies and improprieties" in toxicology studies conducted by Industrial Biotest Laboratories (IBT).44 Problems included "countless deaths of rats and mice that were not reported," "fabricated data tables," and "routine falsification of data."44

IBT was one of the largest laboratories performing tests in support of pesticide registrations.44 About 30 tests on glyphosate and glyphosate-containing products were performed by IBT, including 11 of the 19 chronic toxicology studies.45 A compelling example of the poor quality of IBT data comes from an EPA toxicologist who wrote, "It is also somewhat difficult not to doubt the scientific integrity of a study when the IBT stated that it took specimens from the uteri (of male rabbits) for histopathological examination."46 (Emphasis added.)

In 1991, laboratory fraud returned to the headlines when EPA alleged that Craven Laboratories, a company that performed contract studies for 262 pesticide companies including Monsanto, had falsified test results.47 "Tricks" employed by Craven Labs included "falsifying laboratory notebook entries" and "manually manipulating scientific equipment to produce false reports."48 Roundup residue studies on plums, potatoes, grapes, and sugarbeets were among the tests in question.49

The following year, the owner/president of Craven Laboratories and three employees were indicted on 20 felony counts. A number of other employees agreed to plead guilty on a number of related charges.50 The owner was sentenced to five years in prison and fined $50,000; Craven Labs was fined 15.5 million dollars, and ordered to pay 3.7 million dollars in restitution.48

Although the tests of glyphosate identified as fraudulent have been replaced, these practices cast shadows on the entire pesticide registration process.

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Glyphosate, Part 2:
Human Exposure and Ecological Effects
by Caroline Cox.

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Index

Overview

Residues of the commonly-used herbicide glyphosate have been found in a variety of fruits and vegetables. Residues can be detected long after glyphosate treatments have been made. Lettuce, carrots, and barley planted a year after glyphosate treatment contained residues at harvest.

In California, where reporting of pesticide-caused illnesses is more comprehensive than in other states, glyphosate exposure was the third most commonly-reported cause of pesticide illness among agricultural workers. For landscape maintenance workers, glyphosate ranked highest.

Glyphosate can drift away from the site of its application. Maximum drift distance of 400 to 800 meters (1300-2600 feet) have been measured.

Glyphosate residues in soil have persisted over a year.

Although not expected for an herbicide, glyphosate exposure damages or reduces the population of many animals, including beneficial insects, fish, birds, and earthworms. In some cases glyphosate is directly toxic; for

example, concentrations as low as 10 parts per million can kill fish and 1/20 of typical application rates caused delayed development in earthworms. In other cases, (small mammals and birds, for example) glyphosate reduces populations by damaging the vegetation that provides food and shelter for the animals.

Glyphosate reduces the activity of nitrogen-fixing bacteria. These bacteria transform nitrogen, an essential plant nutrient, into a form that plants can use. Glyphosate reduces the growth of mycorrhizal fungi, beneficial fungi that help plants absorb water and nutrients. Glyphosate also increases the susceptibility of plants to diseases, including Rhizoctonia root rot, take-all disease, and anthracnose.

Glyphosate is a widely-used, broad-spectrum herbicide that is used to kill unwanted plants in a wide variety of agricultural, lawn and garden, aquatic, and forestry situations. It ranks among the top ten herbicides used in the U.S., both in agricultural and nonagricultural situations. Common brand names are Roundup, Rodeo, Accord, and Vision. This is the second part of a summary of glyphosate's hazards Part 1 (JPR 15(3):14-20) discussed the toxicology of glyphosate, its breakdown products, and the other ingredients in glyphosate-containing products. This part discusses human exposure to glyphosate and its ecological effects.

Human Exposure

The most important ways that people are exposed to glyphosate are through workplace exposure (for people who use glyphosate products on the job), eating of contaminated food, exposure caused by off-target movement following application (drift), contact with contaminated soil, and drinking or bathing in contaminated water. The next five sections of this factsheet summarize information about these five routes of exposure. The third section, discussing drift, also covers impacts on plants.

Contamination of Food

Analysis of glyphosate residues is "in general laborious, complex, and costly." (1) For this reason, it is not included in government monitoring of pesticide residues in food. (1) The only information available about contamination of food comes from research situations. Such studies demonstrate several

important points:

* First, glyphosate can be taken up by plants and moved to parts of the plant that are used for food. For example, glyphosate has been found in strawberries, (2) wild blueberries and raspberries, (3) lettuce, carrots, barley,(4) and fish (8,6) following treatment.

* Second, pre-harvest use of glyphosate on wheat (to dry out the grain prior to harvest) results in "significant residues in the grain,"(1) according to the World Health Organization. Bran contains between 2 and 4 times the amount on whole grains. Residues are not lost during baking.(1)_

* Third, glyphosate residues can be found in food long after treatments have been made. For example, lettuce, carrots, and barley contained glyphosate residues at harvest when planted a year after treatment.(4)_

Occupational Exposure

Workers in a variety of occupations are exposed to glyphosate. Researchers have documented exposure for forestry workers in Finland7 and the southeastern U.S., palm plantation workers in Malaysia1 and conifer nursery workers in Mississippi and Oregon.(8) All of these studies generally found low. but consistent, exposure rates.

Physicians, however, paint a different picture. In California, the state with the most comprehensive program for reporting of pesticide-caused illness, glyphosate was the third most commonly-reported cause of pesticide illness among agricultural workers.(9) Among landscape maintenance workers, glyphosate was the most commonly reported cause.(10) (Both these statistics come from reviews of illness reports collected between 1984 and 1990.) Even when glyphosate's extensive use in California is considered, and the illness statistics presented as "number of acute illnesses reported per million pounds used in California," glyphosate ranked twelfth.(9)_

Drift

In general, movement of a pesticide through unwanted drift is "unavoidable."(11) Drift of glyphosate is no exception. Glyphosate drift, however, is a particularly significant problem. Its wide use means that there is a correspondingly large potential for drift.(12) When drift does occur, "damage is likely to be much more extensive and more persistent than with many other herbicides."(13) This is because glyphosate translocates (moves) within plants readily so that even unexposed parts of a plant can be damaged.

Damage to perennial plants (when not exposed to enough glyphosate to kill them) is persistent, with some symptoms lasting several years.(13) In addition, plant susceptibility varies widely. Some wildflowers are almost a hundred times more sensitive than others; small amounts of drift will damage these species.(14)_

A fundamental question about drift is "How far can I expect glyphosate to travel off-site?" Unfortunately, the question is difficult to answer, since drift is "notoriously variable."(18) Factors that increase drift are aerial application techniques, high wind speeds (over 10 kilometers, or 6 miles, per hour), spray nozzles that produce a high proportion of fine droplets, and calm conditions (without enough turbulence to drive the glyphosate droplets onto plant foliage).(18) Drift distances that have been measured for the major application techniques include the following:

* Ground Applications: Between 14 and 78 percent of glyphosate applied as ground sprays moves off-site.(18) Seedling mortality has been demonstrated 20 meters (66 feet) downwind when using a tractor-mounted sprayer. Sensitive species were killed at 40 meters (131 feet).(16) Models indicate that even more sensitive species would be killed at distances approaching 100 meters (328 feet).(14) Glyphosate residues have been measured 400 meters (1312 feet) downwind from ground applications.(17)_

* Helicopter applications: Between 41 and 82 percent of glyphosate applied from helicopters moves off the target site.15 Two studies done in Canada(18),(19) measured glyphosate residues 200 meters (656 feet) from target areas following helicopter applications to forest sites. In both studies, 200 meters was the farthest distance at which samples were taken, so the longest distance glyphosate traveled is not known.(18,(19) A third study (from California) found glyphosate 800 meters (2624 feet) downwind following a helicopter application. Again, this was the farthest distance at which measurements were made. Plant injury was recorded 400 meters (1312 feet) downwind.(17)_

*Fixed-wing aircraft: Long drift distances occur following applications of glyphosate made from fixed-wing airplanes. Three studies on forested sites conducted by Agriculture Canada (the Canadian agricultural ministry) showed that glyphosate was consistently found at the farthest distance from the target areas that measurements were made (200, 300, and 400 meters, or 656, 984, and 1312 feet).20-22 A California study found glyphosate 800 meters downwind of an airplane application. Again, this was the farthest distance at which measurements were made. Plant injury was observed at 100 meters (328 feet). Unlike the first three studies, this study used a grass field as the test site.(17)_

One of the Canadian studies(22) calculated that buffer zones of between 75 and 1200 meters (246 feet - 0.75 miles) would be required to protect nontarget vegetation.

Soil Contamination

Persistence: Glyphosate's persistence in soil varies widely, so giving a simple answer to the question "How long does glyphosate persist in soil?" is not possible. Half-lives (the time required for half of the amount of glyphosate applied to break down or move away) as low as 3 days and as long as 141 days have been measured by glyphosate's manufacturer.(4) Initial degradation (breakdown) is faster than the subsequent degradation of what remains, resulting in long persistence.(23) Long persistence has been measured in the following studies:

55 days on an Oregon Coast Range forestry site(24); 249 days on Finnish agricultural soils(28); between 259 and 296 days on eight Finnish forestry sites(23); 335 days on an Ontario (Canada) forestry site(26); 360 days on 3 British Columbia forestry sites(27); and, from 1 to 3 years on eleven Swedish forestry sites.(27) These are minimum estimates because, in all but two of these studies, glyphosate was detected on the last date samples were analyzed.

Glyphosate is thought to be "readily bound to many soils and clay minerals"1 and therefore "immobile or slightly immobile in many soils."1 This means that the glyphosate will be unlikely to move away from the application site and contaminate water or soil elsewhere. However, a new study(29) paints a different picture. The researchers found that glyphosate bound readily to the four soils studied. However, desorption, when glyphosate unbinds from soil particles, also occurred readily. In one soil, 80 percent of the added glyphosate desorbed in a two hour period. The study concludes that "this herbicide can be extensively mobile in the soil environment.."(29)_

Water Contamination

Based on the prevailing view that glyphosate binds readily to soil particles, it does not have the chemical characteristics of a pesticide that is likely to leach into either ground or surface water.(1) (If it readily desorbs, as described above, this picture would change.) In either case, glyphosate can move into surface water when the soil particles to which it is bound are washed into streams or rivers.(4) How often this happens is not known, because routine monitoring for glyphosate in water is infrequent.(1)_

However, glyphosate has been found in both ground and surface water. Examples include two farm ponds in Ontario, Canada, contaminated by run-off from an agricultural treatment (one pond) and a spill (the other pond)(30); the run-off from a watersheds treated with Roundup during production of no-till corn and fescue (31); contaminated surface water in the Netherlands1; and seven U.S. wells (one in Texas, six in Virginia) contaminated with glyphosate.(32)

Glyphosate's persistence in water is shorter than its persistence in soils. Two Canadian studies found glyphosate persisted 12 to 60 days in pond water following direct application.(33)(34) Glyphosate persists longer in sediments. For example, a study of Accord applied to forest ponds found glyphosate residues in sediment 400 days after application.1 The half-life in pond sediments in a Missouri study was 120 days; persistence was over a year in pond sediments in Michigan and Oregon.(4)

Ecological Effects

Glyphosate can impact many organisms not intended as targets of the herbicide. The next two sections describe both direct mortality and indirect effects, through destruction of food or shelter.

Effects on Nontarget Animals

Beneficial insects: Glyphosate-containing products pose hazards to insects that are economically beneficial because they kill pest insects. **The International Organization for Biological Control found that exposure to freshly dried Roundup killed over 50 percent of three species of beneficial insects: a parasitoid wasp, a lacewing, and a ladybug.35 Over 80 percent of a fourth species, a predatory beetle, was killed.**

Similar impacts on beneficial insects have been shown in field studies. In North Carolina winter wheat fields, populations of large carabid beetles declined after treatment with a commercial glyphosate product and did not recover for 28 days.(36) A study of Roundup treatment of pasture hedgerows in the United Kingdom showed a similar decline in carabid beetles.(37)

Roundup treatment of a Maine clear-cut caused an 89 percent decline in the number of herbivorous (plant-eating) insects. While these are not usually considered beneficial insects, they serve as an important food resource for birds and insect-eating small mammals.(38)

Aquatic insects can also be affected by glyphosate. Midge larvae (important food for breeding waterfowl(39)) are killed by glyphosate in amounts that vary widely. For example, one study found that 55 parts per million (ppm) of glyphosate killed midge larvae(6)while other studies found that 65040 - 560039 ppm of Rodeo (containing glyphosate and water) were required to kill the larvae. Part of the variability is related to water hardness.(39)_

The U.S. Fish and Wildlife Service has identified one endangered species of insect, a longhorn beetle, that would be jeopardized by use of glyphosate.(41)_

Other arthropods: Glyphosate and glyphosate-containing products kill a variety of other arthropods. For example, over 50 percent of test populations of a predatory mite that is an important predator of pest mites was killed by exposure to Roundup.(38) In another laboratory study, Roundup exposure caused a decrease in survival and a decrease in body weight of woodlice. These arthropods are important in humus production and soil aeration.(42) Roundup treatment of pasture hedgerows reduced the number of spiders, probably by killing the plants they preferred for web-spinning.(37) The water flea Daphnia pulex is killed by concentrations of Roundup between 3 and 25 ppm.(6),(34),(44) Young Daphnia are more susceptible than mature individuals, and suspended sediments in the water increased the toxicity.(43) The red swamp crawfish, a commercial species, was killed by 47 ppm of Roundup.(48)_

Fish: Both glyphosate and the commercial products that contain glyphosate are acutely toxic to fish. In general, glyphosate alone is less toxic than the common glyphosate product, Roundup, and other glyphosate products have intermediate toxicity. Part of these differences in toxicity to fish can be explained by the toxicity of the surfactant (detergent-like ingredient) in Roundup. It is about 30 times more toxic to fish than glyphosate itself.(44)_

Acute toxicities of glyphosate vary widely: median lethal concentrations (LC50s; the concentrations killing 50 percent of a population of test animals) from 10 ppm to over 1000 ppm have been reported depending on the species of fish and test conditions.1 In soft water there is little difference between the toxicities of glyphosate and Roundup.

Acute toxicities of Roundup to fish range from an LC50 of 3.2 ppm to an LC50 of 52 ppm.1 Acute toxicities of Rodeo (used with the surfactant X-77 per label recommendations) vary from 120 to 290 ppm.(46)_

Factors important in determining the toxicity of glyphosate or glyphosate-containing products to fish include the following:

* First, different species of fish have different susceptibilities. For example, coho and chinook salmon are more tolerant of glyphosate than pink or chum salmon.(47)_

* Water quality is important: glyphosate in soft water was 20 times more toxic to rainbow trout than was glyphosate in hard water. For Roundup, the reverse is true: it is more toxic in hard water than in soft.(47),(48)_

* Age affects the susceptibility of fish because juveniles are often more susceptible than adults. For example, Roundup was four times more toxic to rainbow trout fry and fingerlings than it was to larger fish.(6)

* Nutrition also can determine toxicity. Hungry fish are more susceptible to glyphosate than fed fish. For example, fed flagfish were 10 times more tolerant of glyphosate than unfed fish.(49)_

* Finally, glyphosate toxicity increases with increased water temperature. In both rainbow trout and bluegills, toxicity about doubled between 7 and 17!C (45 and 63!F).(6) Treatment of riparian areas with glyphosate causes water temperatures to increase for several years following treatment(80) because the herbicide kills shading vegetation. This means that repeated use of glyphosate in a watershed could favor its increased toxicity to fish. In addition, the temperature increase itself could be critical for fish, like juvenile salmon, that are sensitive to water temperature.

Sublethal effects of glyphosate on fish are also significant and occur at low concentrations. Studies of rainbow trout and Tilapia found that concentrations of about 1/2 and 1/3 of the LC50 (respectively) caused erratic swimming.(81),(82) The trout also exhibited labored breathing.(81) Behavioral effects can increase the risk that the fish will be eaten, as well as affecting feeding, migration, and reproduction.(82)_

Birds: Glyphosate is acutely toxic to birds, but only in large amounts. The LC50, the amount in food that kills 50 percent of a population of test animals, is often above 4000 milligrams per kilogram of food.(1)_

Glyphosate also has indirect impacts on birds. Because glyphosate kills plants, its use creates a dramatic change in the structure of the plant community. This affects bird populations, since the birds depend on the plants for food, shelter, and nest support.

For example, a study of four glyphosate-treated clear-cuts (and an unsprayed control plot) in Nova Scotia found that the densities of the two most common species of birds (white-throated sparrow and common yellowthroat) decreased for two years after glyphosate treatment. By the fourth year post-spray,

densities had returned to normal for these two species. However, the unsprayed plot had by then been colonized by new species of birds (warblers, vireos, and a hummingbird). These species did not appear on the sprayed plots.(83)_

An earlier three year study of songbird abundance following glyphosate treatment of clear-cuts in Maine forests showed similar results. Abundance's of the total number of birds (Figure 2) and three common species decreased. The decrease in bird abundance was correlated with decrease in the diversity of the habitat.(84)_

Black grouse avoided glyphosate-treated clear-cuts in Norway for several years after treatment.(88) Researchers recommended that the herbicide not be used near grouse courtship areas Small mammals: In field studies, small mammals have also been indirectly affected when glyphosate kills the vegetation they (or their prey) use for food or shelter. This was first shown in studies of clear-cuts in Maine.(38) Insect-eating shrews declined for three years post-treatment; plant-eating voles declined for two. A second study in Maine(86) found similar results for voles, but not shrews. A British Columbia study found that deer mice populations were dramatically (83 percent) lower following glyphosate treatment.(87) While some other studies have found no affect on mice, this may have occurred because treated areas were small.1 This suggests that effects are more severe when large areas are treated. In Norway, there was a "strong reduction" in use of sprayed clear-cuts by mountain hare.(88)_

Earthworms: A study of the most common earthworm found in agricultural soils in New Zealand showed that glyphosate significantly affects growth and survival of earthworms. Repeated biweekly applications of low rates of glyphosate (1/20 of typical rates) caused a reduction in growth, an increase in the time to maturity, and an increase in mortality.(89)_

Effects on Nontarget Plants

As a broad-spectrum herbicide, glyphosate has potent acutely toxic effects on most plant species. However, there are other kinds of serious effects. These include effects on endangered species, reduction in the ability to fix nitrogen, increased susceptibility to plant diseases, and reduction in the activity of mycorrhizal fungi.

Endangered species: Because essentially all plants are susceptible to glyphosate-caused damage or mortality, glyphosate can seriously impact endangered plant species. The U.S. Fish and Wildlife Service has identified 74 endangered plant species that it believes could be jeopardized by use of

glyphosate. This list is based on the use of glyphosate on 9 crops, and does not include over 50 other uses.41Nitrogen fixation: Nitrogen is important because of its "near omnipresence" in membranes, proteins, and genetic material of living things. Most living things cannot use nitrogen in its common form and instead use ammonia and nitrates, much rarer compounds. The processes by which ammonia and nitrates are created are called nitrogen fixation and nitrification. They are carried out by certain bacteria.(60)_

A number of studies (from Iowa,(61) Australia,(62) eastern Canada,(63) and Ontario (Canada)(64),(68)) have shown that commercial glyphosate products can reduce nitrogen-fixing or nitrification activity of soils. The amount of glyphosate that produces inhibitory effects varies from 262 to 200063 ppm. Effects can be persistent; the formation of nitrogen-fixing nodules on clover roots was inhibited 120 days after treatment. (62)

In addition, tests of cultured nitrogen-fixing bacteria have also shown that glyphosate inhibits nitrogen-fixation. These studies included the nitrogen-fixing species in roots of soybeans(66) and clover.(67),(68)_

Given the importance of nitrogen-fixation to agriculture, more research is crucial. Mycorrhizal fungi: Mycorrhizal fungi are beneficial fungi that live in and around plant roots. They help plants absorb nutrients and water and can protect them from cold and drought.(69) Glyphosate is toxic to many species of mycorrhizal fungi. Effects, mostly growth inhibition, have been observed at concentrations between 1 and 100 ppm.(70),(73)

Plant diseases: Glyphosate treatment increases the susceptibility of crop plants to a number of diseases. For example, glyphosate reduced the ability of bean plants to defend themselves against the disease anthracnose.(74)_

Glyphosate increased the growth of take-all disease in soil from a wheat field. In addition, the proportion of soil fungi which was antagonistic to the take-all fungus decreased.(78) Bean seedlings also survived glyphosate treatment when grown on sterile soil, but not when grown on normal (not sterilized) soil.(76) Spraying of Roundup prior to planting barley increased the severity of Rhizoctonia root rot and decreased barley yield.(77) In addition, Roundup injection of lodgepole pine inhibited the defensive response of the tree to blue stain fungus.(78)

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● HERBICIDE FACTSHEET

GLYPHOSATE (ROUNDUP)

Glyphosate is a broad-spectrum herbicide widely used to kill unwanted plants both in agriculture and in nonagricultural landscapes. Estimated use in the U.S. is between 38 and 48 million pounds per year. Most glyphosate-containing products are either made or used with a surfactant, chemicals that help glyphosate to penetrate plant cells.

Glyphosate-containing products are acutely toxic to animals, including humans. Symptoms include eye and skin irritation, headache, nausea, numbness, elevated blood pressure, and heart palpitations. The surfactant used in a common glyphosate product (Roundup) is more acutely toxic than glyphosate itself; the combination of the two is yet more toxic.

Given the marketing of glyphosate herbicides as benign, it is striking that laboratory studies have found adverse effects in all standard categories of laboratory toxicology testing. These include medium-term toxicity (salivary gland lesions), long-term toxicity (inflamed stomach linings), genetic damage (in human blood cells), effects on reproduction (reduced sperm counts in rats; increased frequency of abnormal sperm in rabbits), and carcinogenicity (increased frequency of liver tumors in male rats and thyroid cancer in female rats).

In studies of people (mostly farmers) exposed to glyphosate herbicides, exposure is associated with an increased risk of miscarriages, premature birth, and the cancer non-Hodgkin's lymphoma.

Glyphosate has been called "extremely persistent" by the U.S. Environmental Protection Agency, and half lives of over 100 days have been measured in field tests in Iowa and New York. Glyphosate has been found in streams following agricultural, urban, and forestry applications.

Glyphosate treatment has reduced populations of beneficial insects, birds, and small mammals by destroying vegetation on which they depend for food and shelter.

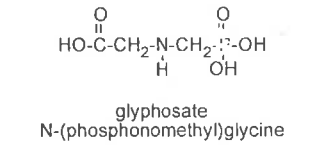
In laboratory tests, glyphosate increased plants' susceptibility to disease and reduced the growth of nitrogen-fixing bacteria.

BY CAROLINE COX

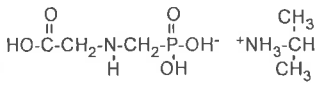
Described by their manufacturer as pesticides of "low toxicity and environmental friendliness,"[1] glyphosate-based herbicides can seem like a silver bullet when dealing with unwanted vegetation. However, glyphosate poses a variety of health and environmental hazards. The following article is a summary of those hazards.

Glyphosate, N-(phosphonomethyl) glycine (Figure 1), is a systemic and nonselective herbicide used to kill broadleaved, grass, and sedge species.[2] It has been registered in the U.S. since 1974 and is used to control weeds in a wide variety of agricultural, urban, lawn and garden, aquatic, and forestry situations.[3] Most glyphosate herbicides contain the isopropylamine salt of glyphosate.[4]

Figure 1
Glyphosate



glyphosate
N-(phosphonomethyl)glycine



isopropylamine salt of glyphosate

Glyphosate products are manufactured by Monsanto Company worldwide. They are marketed under a variety of trade names: Roundup, Rodeo, and Accord are the most common names in the U.S.[2]

Unlike most other herbicides, chemicals which are closely related to glyphosate are not effective herbicides.[5]

Use

Glyphosate is the seventh most commonly used pesticide in U.S. agriculture, the third most commonly used pesticide on industrial and commercial land, and the second most commonly used home and garden pesticide. Estimated annual

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Caroline Cox is JPR's editor.

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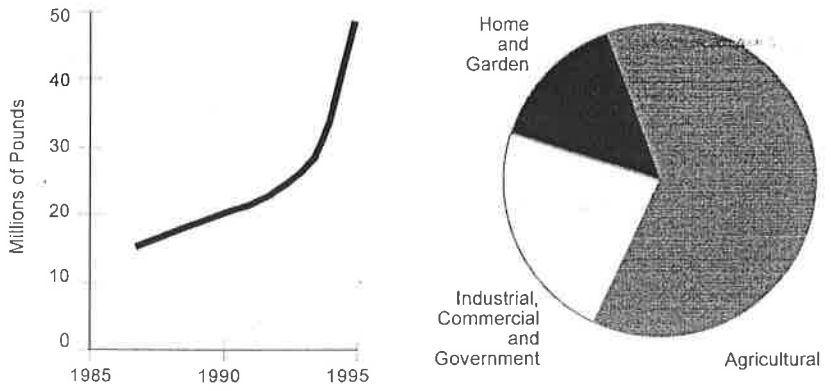
Figure 2
Glyphosate Use in the U.S.



Aspelin, A.L. 1990; 1994; 1997. *Pesticide industry sales and usage: 1988 market estimates; 1992 and 1993 market estimates; 1994 and 1995 market estimates.* U.S. EPA, Office of Prevention, Pesticides and Toxic Substances. Office of Pesticide Programs, Biological and Economic Analysis Division. Washington, D.C.

Use of glyphosate increases about 20 percent each year.

use according to the U.S. Environmental Protection Agency (EPA) is between 38 and 48 million pounds.[6] The largest agricultural uses are in the production of soybeans, corn, hay and pasture, and on fallow land.[7] Glyphosate use is currently (1998) growing at a rate of about 20 percent annually, primarily because of the recent introduction of crops which are genetically engineered to be tolerant of the herbicide.[8] (See Figure 2.)

In the U.S., 25 million applications are made yearly on lawns and in yards.[9]

Mode of Action

Glyphosate's mode of action is "not known at this time,"[4] according to EPA. However, considerable research has established that glyphosate inhibits an enzyme pathway, the shikimic acid pathway, preventing plants from synthesizing three aromatic amino acids. These amino acids are essential for growth and survival of most plants. The key enzyme inhibited by glyphosate is called EPSP synthase.[10] Glyphosate also "may inhibit or repress"[4] two other enzymes, involved in the synthesis of the same amino acids.[4] These enzymes are present in higher plants and microorganisms but not in animals.[10]

Two of the three aromatic amino acids are essential amino acids in the human diet because humans, like all higher animals, lack the shikimic acid pathway, cannot synthesize these amino acids, and rely on their foods to provide these compounds. One is synthesized in animals through another pathway.[11]

Glyphosate can affect plant enzymes not connected with the shikimic acid pathway. In sugar cane, it reduces the activity of one of the enzymes involved in sugar metabolism.[12] It also inhibits a major detoxification enzyme in plants.[13]

Roundup affects enzymes found in mammals. In rats, Roundup decreased the activity of two detoxification enzymes in the liver and an intestinal enzyme.[14]

"Inert" Ingredients in Glyphosate-containing Products

Virtually every pesticide product contains ingredients other than what is called the "active" ingredient(s), the one designed to provide killing action. These ingredients are misleadingly called "inert." The purpose of these "inerts" is to make the product easier to use or more efficient. In general, they are not identified on the labels of pesticide products.

In the case of glyphosate products, many "inerts" have been identified. See "Toxicology of 'Inert' Ingredients of Glyphosate-containing Products," p. 5, for basic information about these "inerts."

Many of the toxicology studies that will be summarized in this factsheet have been conducted using glyphosate, the active ingredient, alone. Some have been conducted with commercial products containing glyphosate and "inert" ingredients. When no testing is done with the product as it is actually used, it is impossible to accurately assess its hazards.

We will discuss both types of studies, and will identify insofar as is possible what material was used in each study.

Acute Toxicity to Laboratory Animals

Glyphosate's acute oral median lethal dose (the dose that causes death in 50 percent of a population of test animals; LD_{50}) in rats is greater than 4,320 milligrams per kilogram (mg/kg) of body weight. This places the herbicide in Toxicity Category III (Caution).[4] Its acute dermal toxicity (dermal LD_{50}) in rabbits is greater than 2,000 mg/kg of body weight, also Toxicity Category III.[4]

Commercial glyphosate herbicides are more acutely toxic than glyphosate. The amount of Roundup (containing glyphosate and the surfactant POEA) required to kill rats is about 1/3 the amount of glyphosate alone.[15] Roundup is also more acutely toxic than POEA.[15]

Glyphosate-containing products are more toxic via inhalation than orally. Inhalation of Roundup by rats caused "signs of toxicity in all test groups,"[16] even at the lowest concentration tested. These signs included gasping, congested eyes, reduced activity,[17] and body weight loss.[16] Lungs were red or blood-congested.[17] The dose required to cause lung damage and mortality following pulmonary administration of two Roundup products and POEA (when forced into the trachea, the tube carrying air into the lungs) was only

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JOURNAL OF PESTICIDE REFORM/ FALL 1998 • VOL.18, NO. 3
UPDATED 10/00

1/10 the dose causing damage orally.[15,18]

Effects on the Circulatory System: When dogs were given intravenous injections of glyphosate, POEA, or Roundup so that blood concentrations were approximately those found in humans who ingested glyphosate, glyphosate increased the ability of the heart muscle to contract. POEA reduced the output of the heart and the pressure in the arteries. Roundup caused cardiac depression.[19]

Eye Irritation: NCAP surveyed eye hazards listed on material safety data sheets for 25 glyphosate-containing products. One of the products is "severely irritating,"[20] 4 cause "substantial but temporary eye injury,"[21-24] 8 "cause eye irritation,"[25-32] 5 "may cause eye irritation,"[33-37] 1 is "moderately irritating,"[38] and 3 are "slightly irritating."[39-41] The other three products require addition of a surfactant (wetting agent) before use,[42-44] and the surfactant sold by glyphosate's manufacturer for this purpose "causes eye burns."[45]

Skin Irritation: Glyphosate is classified as a slightly irritating to skin. Roundup is a "moderate skin irritant," and recovery can take over two weeks.[16]

Acute Toxicity to Humans

The acute toxicity of glyphosate products to humans was first publicized by physicians in Japan who studied 56 suicide attempts; nine cases were fatal. Symptoms included intestinal pain, vomiting, excess fluid in the lungs, pneumonia, clouding of consciousness, and destruction of red blood cells.[66] They calculated that the fatal cases ingested on average about 200 milliliters (3/4 of a cup). They believed that POEA was the cause of Roundup's toxicity.[66] More recent reviews of poisoning incidents have found similar symptoms, as well as lung dysfunction,[67-69], erosion of the gastrointestinal tract,[67,69] abnormal electrocardiograms,[69] low blood pressure,[67,69] kidney damage,[67,68,70] , and damage to the larynx.[71]

Smaller amounts of Roundup cause adverse effects, usually skin or eye irritation as well as some of the symptoms

TOXICOLOGY OF "INERT" INGREDIENTS IN GLYPHOSATE-CONTAINING PRODUCTS

Three glyphosate products contain **ammonium sulfate.**[29,30,32] It causes eye irritation, nausea and diarrhea, and may cause allergic respiratory reactions. Prolonged exposure can cause permanent eye damage.[46]

One glyphosate product contains **benzisothiazolone.**[47] It causes eczema, skin irritation,[48] and a light-induced allergic reaction in sensitive people.[49,50]

Four glyphosate products contain **3-iodo-2-propynyl butylcarbamate (IPBC).**[39-41,47] It is severely irritating to eyes and increases the incidence of miscarriages in laboratory tests.[51] It also can cause allergic skin reactions.[52]

One glyphosate product contains **isobutane.**[30] It causes nausea, nervous system depression, and difficulty breathing. It is a severe fire hazard.[53]

One glyphosate product contains **methyl pyrrolidinone.**[20] It causes severe eye irritation.[54] It has caused fetal loss and reduced fetal weights in laboratory animals.[55]

Three glyphosate products contain **pelargonic acid.**[29,30,32] It causes severe eye and skin irritation and may cause respiratory tract irritation.[56]

Nine glyphosate products contain **polyethoxylated tallowamine (POEA).**[21-24,31,35-38] It causes eye burns; skin redness, swelling, and blistering; nausea; and diarrhea.[23,45]

Three glyphosate products contain **potassium hydroxide.**[29,30,32] It causes irreversible eye injury, deep skin ulcers, severe digestive tract burns, and severe irritation of the respiratory tract.[57]

One glyphosate product contains **sodium sulfite.**[34] It may cause eye and skin irritation with vomiting and diarrhea[58] as well as skin allergies.[59] Exposure to small amounts can cause severe allergic reactions.[60]

Three glyphosate products contain **sorbic acid.**[35,36,37] It may cause severe skin irritation, nausea, vomiting, chemical pneumonitis, and sore throat.[61] It also causes allergic reactions.[62,63]

Isopropylamine is used in some Roundup products.[47,64] It is "extremely destructive to tissue of the mucous membranes and upper respiratory tract."[65] Symptoms of exposure are wheezing, laryngitis, headache, and nausea.[65]

Table 1
Symptoms Following Unintentional Exposure to Glyphosate Herbicides

eye irritation
painful eyes
burning eyes
blurred vision
swollen eye, face, joints
facial numbness
burning sensation on skin
itchy skin
tingling skin
recurrent eczema
blisters
skin rash
rapid heartbeat
heart palpitations
elevated blood pressure
chest pains
congestion
coughing
headache
nausea

Temple, W.A. and N.A. Smith. 1992. Glyphosate herbicide poisoning experience in New Zealand. *N.Z. Med. J.* 105:173-174.

Calif. EPA. Dept. of Pesticide Regulation. 1998. Case reports received by the California Pesticide Illness Surveillance Program in which health effects were attributed to glyphosate, 1993-1995. Unpublished report.

JOURNAL OF PESTICIDE REFORM/FALL 1998 • VOL.18, NO. 3
UPDATED 10/00

listed above. (See Table 1.) For example, rubbing of Roundup in an eye caused eye and lid swelling, rapid heartbeat and elevated blood pressure. Wiping the face after touching leaky spray equipment caused swelling of the face. Accidental drenching with horticultural Roundup caused eczema of the hands and arms lasting two months.[68] A spill resulted in dizziness, fever, nausea, palpitations, and sore throat.[72]

Toxicology Overview

Glyphosate is often portrayed as toxicologically benign: "extensive investigations strongly support the conclusion that glyphosate has a very low level of toxicity..."[73] NCAP's review of glyphosate's toxicology comes to a different conclusion. Adverse effects have been identified in each standard category of testing (subchronic, chronic, carcinogenicity, mutagenicity, and reproduction). NCAP's review has been challenged by the assertion that these effects were found because standard test protocols *require* finding adverse effects at the highest dose tested. However, the following five sections of this article summarize adverse effects did *not* result from this requirement: they were all found at less than the highest dose tested. (The few exceptions are clearly identified.)

Subchronic Toxicity

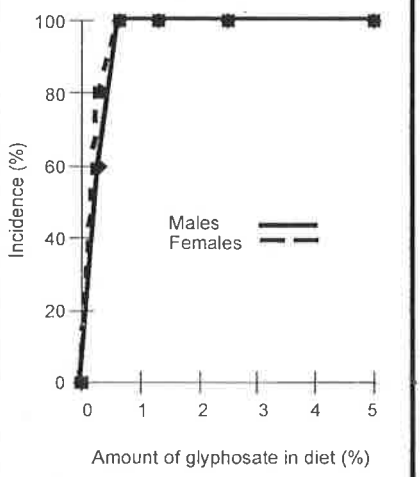
In subchronic (medium term) studies of rats and mice done by the National Toxicology Program (NTP), microscopic salivary gland lesions were found in all doses tested in rats (200 - 3400 mg/kg per day) and in all but the lowest dose tested in mice (1,000-12,000 mg/kg per day). (See Figure 3.) A follow-up study by NTP found that the mechanism by which glyphosate caused these lesions involved the hormone adrenalin.[74]

The NTP study also found increases in two liver enzymes at all but the two lowest doses tested. Other effects found in at least two doses in this study were reduced weight gain in rats and mice; diarrhea in rats; and changes in kidney and liver weights in male rats and mice.[74]

Another subchronic laboratory test found that blood levels of potassium and phosphorus in rats increased at all doses tested (60-1600 mg/kg/day).[4]

Glyphosate-containing products are more toxic than glyphosate in subchronic tests. In a 7 day study with calves, 790 mg/kg per day of Roundup caused pneumonia, and death of 1/3 of the animals tested. At lower doses decreased food intake and diarrhea were observed.[2]

Figure 3
Salivary Gland Lesions in Rats Fed Glyphosate



U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health. 1992. NTP technical report on toxicity studies of glyphosate (CAS No. 1071-83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. Research Triangle Park, NC: National Toxicology Program.

Glyphosate causes salivary gland lesions in rats, mediated by the hormone adrenalin.

Chronic Toxicity

Glyphosate is also toxic in long-term studies. At all but the lowest dose tested, excessive cell division in the urinary bladder occurred in male mice[2] and inflammation of the stomach lining occurred in both sexes of rats.[2]

Carcinogenicity

A recent Swedish study of hairy cell leukemia (HCL), a form of the cancer non-Hodgkin's lymphoma, found that people who were occupationally exposed to glyphosate herbicides had a threefold higher risk of HCL. A similar study of people with non-Hodgkin's lymphoma found exposure to glyphosate herbicides was associated with an increase in risk of about the same size.[74ab]

The publicly available laboratory studies of glyphosate's ability to cause cancer were all conducted by or for its manufacturer.[2] The first carcinogenicity study submitted to EPA (1981) found an increase in testicular tumors in male rats at the highest dose tested as well as an increase in the frequency of a thyroid cancer in females. Both results occurred at the highest dose tested (30 mg/kg of body weight per day).[75,76] The second study (1983) found an increasing trend in the frequency of a rare kidney tumor in male mice.[77] The most recent study (1990) found an increase in pancreas and liver tumors in male rats together with an increase of the same thyroid cancer found in the 1983 study in females.[78]

All of these increases in tumor or cancer incidence are "not considered compound-related"[78] according to EPA (This means that EPA did not consider glyphosate the cause of the tumors.) For the testicular tumors, EPA accepted the interpretation of an industry pathologist who said that the incidence in treated groups (12 percent) was similar to those observed (4.5 percent) in other rats *not* fed glyphosate.[78] For the thyroid cancer, EPA stated that it was not possible to distinguish between cancers and tumors of this type, so that the two should be considered together. The combined data are not statistically significant.[76] For the kidney tumors, the manufacturer reexamined the tissue and found an additional tumor in untreated mice so that statistical significance was lost. This was despite the opinion of EPA's pathologist that the lesion in question was not really a tumor.[77] For the pancreatic tumors, EPA stated that there was no dose-related trend. For the liver and thyroid tumors, EPA stated that pairwise comparisons between treated and untreated animals were not statistically significant.[78]

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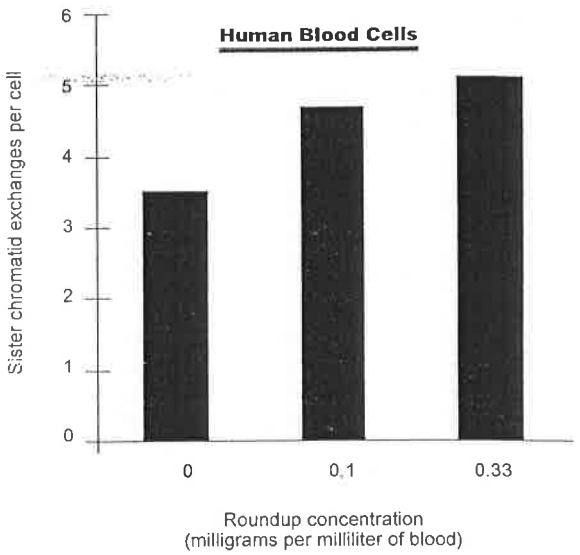
Figure 4
Genetic Damage Caused by Roundup

Peluso, M. et al. 1998. ^{32}P-Postlabeling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ. Molec. Mutag.* 31:55-59.



Bolognesi, C. et al. 1997. Genotoxic activity of glyphosate and its technical formulation Roundup. *J. Agric. Food Chem.* 45:1957-1962.

Roundup causes genetic damage in laboratory animals and in human blood cells.

EPA concluded that glyphosate should be classified as Group E, "evidence of non-carcinogenicity for humans."[78] They added that this classification "should not be interpreted as a definitive conclusion."[78] The cancer tests leave many questions unanswered. Concerning one of the carcinogenicity studies, an EPA statistician wrote, "Viewpoint is a key issue. Our viewpoint is one of protecting the public health when we see suspicious data."[79] Unfortunately, EPA has not taken that viewpoint in its assessment of glyphosate's cancer-causing potential.

There are no publicly available laboratory studies of the carcinogenicity of Roundup or other glyphosate-containing products.

Mutagenicity

Although glyphosate's manufacturer describes "a large battery of assays"[80] showing that glyphosate does not cause genetic damage,[80] other studies have shown that both glyphosate and glyphosate products are mutagenic. Glyphosate-containing products are more potent mutagens than glyphosate.[81] The studies include the following:

- In fruit flies, Roundup and Pondmaster (an aquatic herbicide consisting of glyphosate and a trade secret surfactant[82]) both increased the frequency of sex-linked, recessive lethal mutations. (These are mutations that are usually visible only in males.) Only a single concentration was tested in this study.[83]
- A study of human lymphocytes (a type of white blood cell) showed an increase in the frequency of sister chromatid exchanges following exposure to the lowest dose tested of Roundup.[84] (Sister chromatid exchanges are exchanges of genetic material during cell division between members of a chromosome pair. They result from point mutations.) A 1997 study of human lymphocytes (see Figure 4) found similar results with Roundup (at both doses tested) and with glyphosate (at all but the lowest dose tested).[81]
- In *Salmonella* bacteria, Roundup was weakly mutagenic at two concentrations. In onion root cells, Roundup caused an increase in chromosome aberrations, also at two concentrations.[85]
- In mice injected with Roundup, the frequency of DNA adducts (the binding to genetic material of reactive molecules that lead to mutations) in the liver and kidney increased at all three doses tested.[86] (See Figure 4.)
- In another study of mice injected with glyphosate and Roundup, the frequency of chromosome damage and DNA damage increased in bone marrow, liver, and kidney. (Only a single concentration was tested in this study.)[81]

Reproductive Effects

Glyphosate exposure has been linked to reproductive problems in humans. A study in Ontario, Canada, found that fathers' use of glyphosate was associated with an increase in miscarriages and premature births in farm families.[87] (See Figure 5.) In addition, a case report from the University of California discussed a student athlete who suffered abnormally

frequent menstruation when she competed at tracks where glyphosate had been used.[88]

Laboratory studies have also demonstrated a number of effects of glyphosate on reproduction.

In rats, glyphosate reduced sperm counts at the two highest doses tested. (See Figure 5.) In male rabbits, glyphosate at doses of 1/10 and 1/100 of the LD_{50} increased the frequency of abnormal and dead sperm.[89]

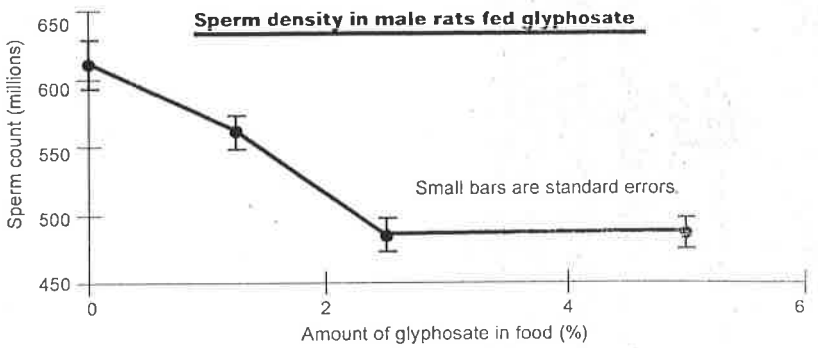
Using cells taken from Leydig cell testicular tumors in mice, researchers from Texas Tech University showed that exposure to Roundup (but not glyphosate alone) caused a decrease in the production of sex hormones. Specifically, Roundup inhibited the expression of a protein that carries cholesterol (the molecule from which sex hormones are made) to the site where these hormones are synthesized. Lacking necessary amounts of cholesterol, the testicle cells' production of sex hormones decreased about 90 percent.[89a]

In a study of female rabbits, glyphosate caused a decrease in fetal weight in all treated groups.[90]

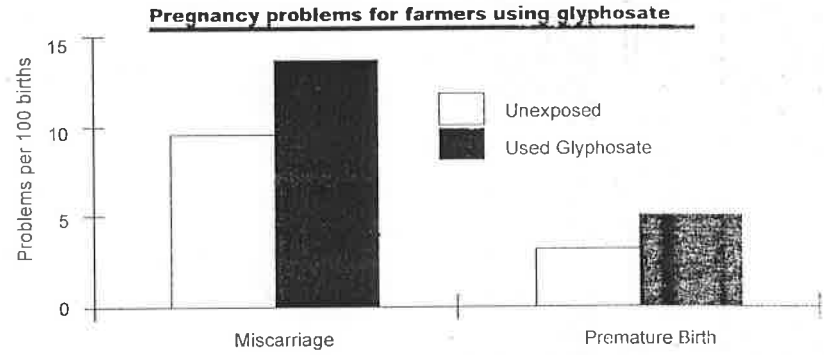
Toxicology of Glyphosate's Major Metabolite

In general, studies of the breakdown of glyphosate find only one metabolite, aminomethylphosphonic acid (AMPA).[2] Although AMPA has low acute toxicity (its LD_{50} is 8,300 mg/kg of body weight in rats),[16] it causes a variety of toxicological problems. In subchronic tests on rats, AMPA caused an increase in the activity of an enzyme, lactic dehydrogenase, in both sexes; a decrease in liver weights in males at all doses tested; and excessive cell division in the lining of the urinary bladder in both sexes.[16] AMPA is more persistent than glyphosate; studies in eight states found that the half-life in soil (the time required for half of the original concentration of a compound to break down or dissipate) was between 119 and 958 days.[2] AMPA has been found in lettuce and barley planted a year after glyphosate treatment.[90a]

Figure 5
Effects of Glyphosate on Male Reproductive Success



U.S. Dept. of Health and Human Services. Public Health Serv. National Inst. Health. 1992. NTP technical report on toxicity studies of glyphosate (CAS No. 1071-83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. Research Triangle Park, NC: National Toxicology Program.



Savitz, D.A. et al. 1997. Male pesticide exposure and pregnancy outcome. *Am. J. Epidemiol* 146:1025-1036.

Glyphosate exposure is associated with reproductive problems in both laboratory animals and farmers.

Quality of Laboratory Testing

Tests done on glyphosate to meet registration requirements have been associated with fraudulent practices.

Laboratory fraud first made headlines in 1983 when EPA publicly announced that a 1976 audit had discovered "serious deficiencies and improprieties" in studies conducted by Industrial Biotest Laboratories (IBT).[91] Problems included "countless deaths of rats and mice" and "routine falsification of data."[91]

IBT was one of the largest laboratories performing tests in support of pesticide registrations.[91] About 30 tests on glyphosate and glyphosate-containing products were performed by IBT, including 11 of the 19 chronic toxicology studies.[92] A compelling example of the poor quality of IBT data comes from an EPA toxicologist who wrote, "It is also somewhat difficult not to doubt the scientific integrity of a study when the IBT stated that it took specimens from the *uteri* (of *male* rabbits) for histopathological examination."[93] (Emphasis added.)

In 1991, EPA alleged that Craven Laboratories, a company that performed studies for 262 pesticide companies including Monsanto, had falsified tests.[94] "Tricks" employed by Craven Labs included "falsifying laboratory notebook entries" and "manually manipulating sci-

JOURNAL OF PESTICIDE REFORM/FALL 1998 • VOL.18, NO. 3
UPDATED 10/00

entific equipment to produce false reports."[95] Roundup residue studies on plums, potatoes, grapes, and sugarbeets were among the tests in question.[96]

The following year, the owner of Craven Labs and three employees were indicted on 20 felony counts.[97] The owner was sentenced to five years in prison and fined $50,000; Craven Labs was fined 15.5 million dollars, and ordered to pay 3.7 million dollars in restitution.[95]

Although the tests of glyphosate identified as fraudulent have been replaced, this fraud casts shadows on the entire pesticide registration process.

Illegal Advertising

In 1996, Monsanto Co. negotiated an agreement with the New York attorney-general that required Monsanto to stop making certain health and environmental claims in ads for glyphosate products and pay the attorney general $50,000 in costs.[98] Claims that glyphosate products are "safer than table salt,"[98] safe for people, pets, and the environment, and degrade "soon after application"[98] were challenged by the attorney-general because they are in violation of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the national pesticide law.[98] According to the attorney-general, Monsanto had engaged in "false and misleading" advertising.[98]

In 1998, Monsanto Co. negotiated a similar agreement with the New York attorney-general about a different advertisement. The attorney-general found that the advertisement featuring a horticulturist from the San Diego Zoo also was "false and misleading" because it implied to consumers that Roundup could be used (contrary to label directions) in and around water.[98a] Monsanto paid $75,000 in costs.[98a]

EPA made a similar determination about Roundup ads in 1998, finding that they contained "false and misleading"[99] claims and were in violation of FIFRA. However, EPA took no action and did not even notify Monsanto Co. about the determination because two years had elapsed between the time that the ads were submitted to EPA and the time that EPA made the determination.[99]

Human Exposure

People are exposed to glyphosate through workplace exposure (for people who use glyphosate products on the job), eating of contaminated food, exposure caused by off-target movement following application (drift), contact with contaminated soil, and drinking or bathing in contaminated water. The next five sections of this factsheet summarize information about these five routes of exposure. The third section, discussing drift, also covers impacts on plants.

Contamination of Food

Analysis of glyphosate residues is "in general laborious, complex, and costly."[2]

"Glyphosate's manufacturer reported that drift from a ground application in Minnesota damaged 25 acres of corn, and the Washington Department of Agriculture reported damage to 30 acres of onions from a ground application of a glyphosate herbicide."

For this reason, it is not included in government monitoring of pesticide residues in food.[2] The only information available about contamination of food comes from research studies.

Monsanto's studies of residues in food crops found glyphosate in lettuce over five months after treatment (the lettuce was planted four months after treatment). Monsanto also found glyphosate in barley over four months after treatment (the barley was planted one month after treatment).[90a]

"Significant residues,"[2] according to the World Health Organization, have been identified from pre-harvest use of glyphosate on wheat (to dry out the grain). Bran contains between 2 and 4 times the amount on whole grains. Residues are not lost during baking.[2]

Occupational Exposure

In California, the state with the most comprehensive program for reporting of pesticide-caused illness, glyphosate-containing herbicides were the third most commonly-reported cause of pesticide illness among agricultural workers.[100] Among landscape maintenance workers, glyphosate herbicides were the most commonly reported cause.[101] (Both these statistics come from illness reports collected between 1984 and 1990.) Even when glyphosate's extensive use in California is considered, and the illness statistics presented as "number of acute illnesses reported per million pounds used in California," glyphosate ranked twelfth.[100]

While many of the California reports involve "irritant effects,"[102] mostly to the eyes and skin, NCAP's survey of about 100 reports made in 1993, 1994, and 1995 found that over half of them involved more serious effects: burning of eyes or skin, blurred vision, peeling of skin, nausea, headache, vomiting, diarrhea, chest pain, dizziness, numbness, burning of the genitals, and wheezing.[103]

Other occupational symptoms were observed in a flax milling operation in Great Britain. A study compared the effects of breathing dust from flax treated with Roundup with the effects of dust from untreated flax. Treated dust caused a decrease in lung function and an increase in coughing, and breathlessness.[104]

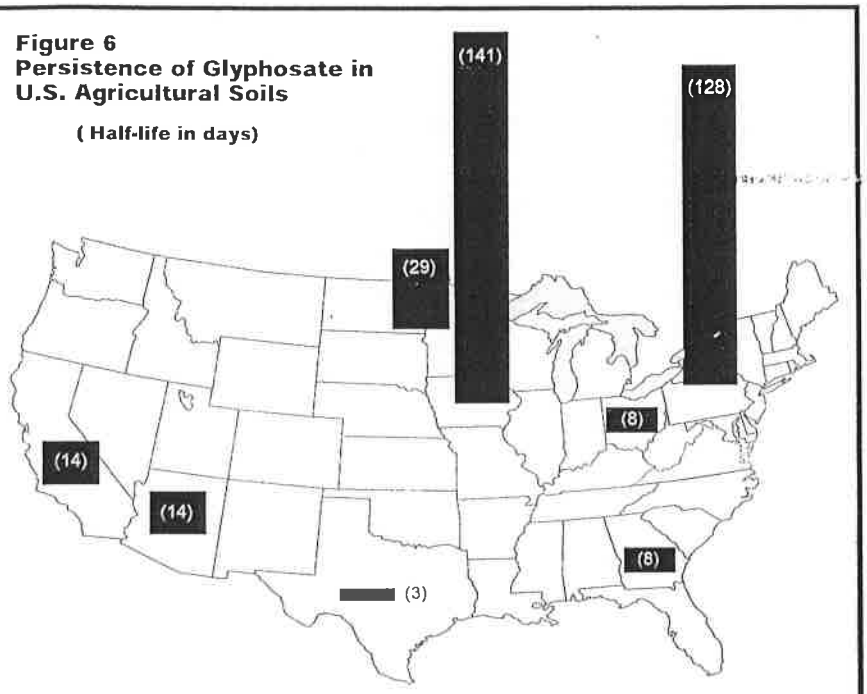
Drift

In general, movement of a pesticide through unwanted drift is "unavoidable."[105] Drift of glyphosate is no exception. Glyphosate drift, however, is particularly significant because drift "dam-

JOURNAL OF PESTICIDE REFORM/FALL 1998 • VOL 18, NO. 3
UPDATED 10/00

Figure 6
Persistence of Glyphosate in U.S. Agricultural Soils

(Half-life in days)



Note: Numbers, as well as the length of the columns, give the half-life, in days, of glyphosate in soil. Half-life is the length of time required for half the applied glyphosate to break down or move out of the test site.

Source: U.S. EPA. Environmental Fate and Effects Division. 1993. Pesticide environmental fate one line summary; Glyphosate. Washington, D.C., May 6.

Glyphosate's persistence in soil varies widely, but its half-life in agricultural soil can be over 4 months.

age is likely to be much more extensive and more persistent than with many other herbicides."[106] This is because glyphosate moves readily within plants so that even unexposed parts of a plant can be damaged. Damage to perennial plants (when not exposed to enough glyphosate to kill them) is persistent, with some symptoms lasting several years.[106] In addition, plant susceptibility varies widely. Some wildflowers are almost a hundred times more sensitive than others; drift in amounts equal to 1/1000 of typical application rates will damage these species.[107]

A simple answer to the question, "How far can I expect glyphosate to travel offsite?" is difficult, since drift is "notoriously variable."[108] However, extensive drift of glyphosate has been measured since the 1970s when a California study found glyphosate 800 m (2600 feet) from aerial and ground applications. Similar drift distances were found for the 8 different spray systems tested in this study.[109]

Drift distances that have been measured more recently for the major application techniques include the following:

• Ground Applications: A study of 15 noncrop plants found seedling mortality (killing about 10 percent of seedlings) for most of the species tested at 20 meters (66 feet) downwind when using a tractor-mounted sprayer. Seedlings of some sensitive species were killed at 40 meters (131 feet).[110] A drift model predicted some native species would be damaged at distances of 80 meters (262 feet).[107] Glyphosate's manufacturer reported that drift from a ground application in Minnesota damaged 25 acres of corn,[111] and the Washington Department of Agriculture reported damage to 30 acres of onions from a ground application of a glyphosate herbicide.[112]

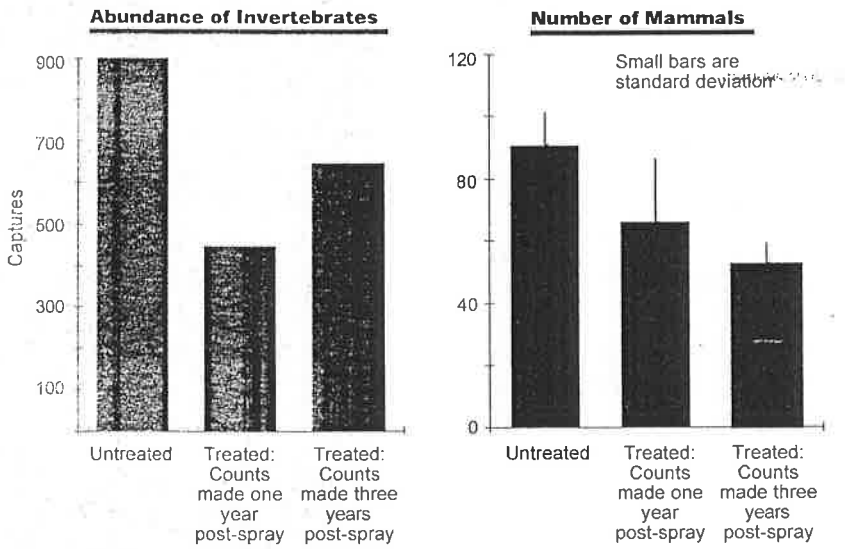
• Helicopter applications: A study done in Canada[113] measured glyphosate residues 200 meters (656 feet) from target areas following helicopter applications to forest sites. In this study, 200 meters was the farthest distance at which samples were taken, so the longest distance glyphosate travelled is not known.

Fixed-wing aircraft: Long drift distances occur following applications of glyphosate made from airplanes. Two studies on forested sites conducted by Agriculture Canada (the Canadian agricultural ministry) showed that glyphosate was found at the farthest distance from the target areas that measurements were made (300 and 400 meters, or 984 and 1312 feet).[114,115] One of these studies[115] calculated that buffer zones of between 75 and 1200 meters (246 feet - 0.75 miles) would be required to protect nontarget vegetation. According to Monsanto, drift from single aerial applications of glyphosate has been extensive enough to damage 1000 trees in one case,[116] 250 acres of corn in another,[117] and 155 acres of tomatoes in a third incident.[118]

Persistence and Movement in Soil

Glyphosate's persistence in soil varies widely, so giving a simple answer to the question "How long does glyphosate persist in soil?" is not possible. Half-lives (the time required for half of the amount of glyphosate applied to break down or move away) as low as 3 days (in Texas) and as long as 141 days (in Iowa) have been measured by glyphosate's manufacturer.[119] (See Figure 6.) Initial degradation (breakdown) is faster than the subsequent degradation of what remains.[120] Long persistence has been measured in the following studies: 55 days on an Oregon Coast Range forestry site[121]; 249 days on Finnish agricultural soils[122]; between 259 and 296 days on eight Finnish forestry sites[120]; 335 days on an Ontario (Canada) forestry site[123]; 360 days on 3 British Columbia forestry sites[124]; and, from 1 to 3 years on eleven Swedish forestry sites.[125] EPA's Ecologi-

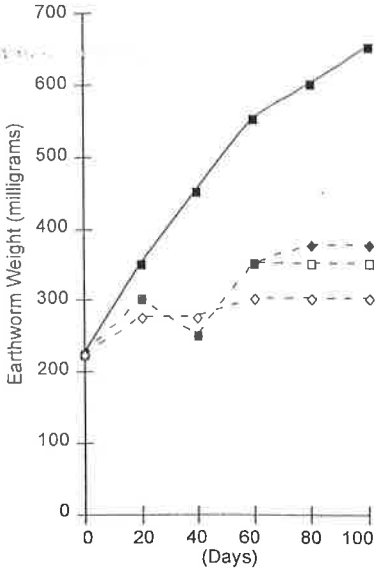
Figure 7
Impacts of Glyphosate on Nontarget Animals on Maine Clear-cuts



Santillo, D.J., D.M. Leslie, and P.W. Brown. 1989. Responses of small mammals and habitat to glyphosate application on clearcuts. *J. Wildl. Manage.* 53(1):164-172.

Glyphosate treatment reduced invertebrate and small mammal populations for up to 3 years.

Figure 8
Effect of Glyphosate on the Growth of Earthworms



Springett, J.A. and R.A.J. Gray. 1992. Effect of repeated low doses of biocides on the earthworm *Aporrectodea caliginosa* in laboratory culture. *Soil Biol. Biochem.* 24(12):1739-1744.

Repeated applications of glyphosate reduce the growth of earthworms.

cal Effect's Branch wrote, "In summary, this herbicide is extremely persistent under typical application conditions."[126]

Glyphosate is thought to be "tightly complexed [bound] by most soils"[127] and therefore "in most soils, glyphosate is essentially immobile."[127] This means that the glyphosate will be unlikely to contaminate water or soil away from the application site. However, this binding to soil is "reversible." For example, one study found that glyphosate bound readily to four different soils. However, desorption, when glyphosate unbinds from soil particles, also occurred readily. In one soil, 80 percent of the added glyphosate desorbed in a two hour period. The study concluded that "this herbicide can be extensively mobile in the soil...."[128]

Water Contamination

When glyphosate binds readily to soil particles, it does not have the chemical characteristics of a pesticide that is likely to leach into water.[2] (When it readily desorbs, as described above, this changes.) However, glyphosate can move into surface water when the soil particles to which it is bound are washed into streams or rivers.[4] How often this happens is not known, because routine monitoring for glyphosate in water is infrequent.[2]

Glyphosate has been found in both ground and surface water. Examples include farm ponds in Ontario, Canada, contaminated by runoff from an agricultural treatment and a spill[129]; the runoff from a watersheds treated with Roundup during production of no-till corn and fescue[130]; contaminated surface water in the Netherlands[2]; seven U.S. wells (one in Texas, six in Virginia) contaminated with glyphosate[131]; contaminated forest streams in Oregon and Washington[132,133]; contaminated streams near Puget Sound, Washington[134]; and contaminated wells under electrical substations treated with glyphosate.[135]

Glyphosate's persistence in water is shorter than its persistence in soils. Two Canadian studies found glyphosate persisted 12 to 60 days in pond water.[136,137] Glyphosate persists longer in pond sediments (mud at the bottom of a pond). For example, the half-life in pond sediments in a Missouri study was 120 days; persistence was over a year in pond sediments in Michigan and Oregon.[4]

Ecological Effects

Glyphosate can impact many organisms not intended as targets of the herbicide. The next two sections describe both direct mortality and indirect effects, through destruction of food or shelter.

Effects on Nontarget Animals

Beneficial insects: Beneficial insects kill other species that are agricultural pests. The International Organization for Biological Control found that exposure to freshly dried Roundup killed over 50

percent of three species of beneficial insects: a parasitoid wasp, a lacewing, and a ladybug. Over 80 percent of a fourth species, a predatory beetle, was killed.[138]

Impacts on beneficial insects have also been shown in field studies, probably due to destruction of their habitat by the herbicide. In North Carolina wheat fields, populations of large carabid beetles declined after treatment with a glyphosate product and did not recover for 28 days.[139] A study of Roundup treatment of hedgerows in the United Kingdom also showed a decline in carabid beetles.[140]

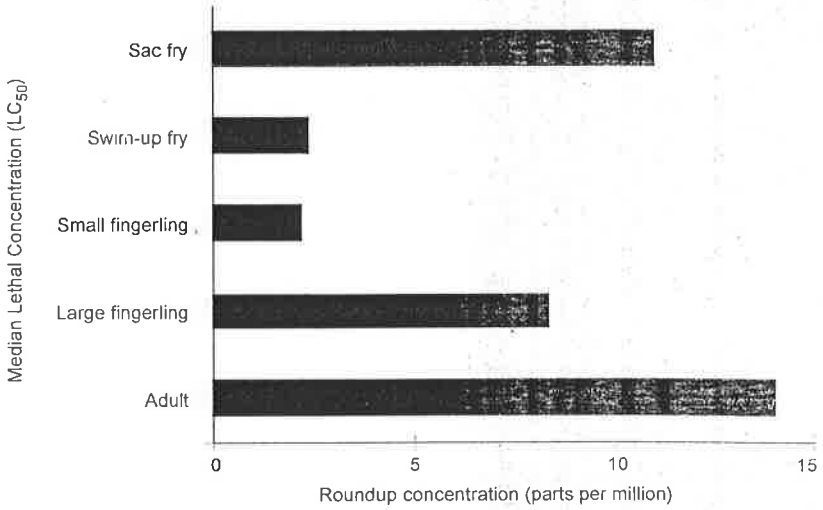
Other insects: Roundup treatment of a Maine clear-cut caused an 89 percent decline in the number of herbivorous (plant-eating) insects because of the destruction of the vegetation on which they live and feed. (See Figure 7.) These insects serve as food resources for birds and insect-eating small mammals.[141]

The U.S. Fish and Wildlife Service has identified one endangered insect, a longhorn beetle, that would be jeopardized by use of glyphosate herbicides.[142]

Other arthropods: Glyphosate and glyphosate-containing products kill a variety of other arthropods. For example, over 50 percent of test populations of a beneficial predatory mite were killed by exposure to Roundup.[138] In another laboratory study, Roundup exposure caused a decrease in survival and a decrease in body weight of woodlice. These arthropods are important in humus production and soil aeration.[143] Roundup treatment of hedgerows reduced the number of spiders, probably by killing the plants they preferred for web-spinning.[140] The water flea *Daphnia pulex* is killed by concentrations of Roundup between 3 and 25 ppm.[144-146] Young *Daphnia* are more susceptible than mature individuals.[145] The red swamp crawfish, a commercial species, was killed by 47 ppm of Roundup.[147]

Earthworms: A study of the most common earthworm found in agricultural soils in New Zealand showed that repeated applications of glyphosate significantly affect growth and survival of earthworms. Biweekly applications of low rates of glyphosate (1/20 of typical rates) caused a reduction in growth (see Figure 8), an increase in the time to maturity, and an increase in mortality.[148]

Figure 9
Toxicity of Roundup to Rainbow Trout of Different Ages



Folmar, L.C., H.O. Sanders, and A.M. Julin. 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* 8:269-278.

Young rainbow trout (swim-up fry and small fingerlings) are more susceptible to Roundup than adult rainbow trout.

Fish: Both glyphosate and the commercial products that contain glyphosate are acutely toxic to fish. In general, glyphosate alone is less toxic than the common glyphosate product, Roundup, and other glyphosate products have intermediate toxicity. Part of these differences can be explained by the toxicity of the surfactant (detergent-like ingredient) in Roundup. It is 20 to 70 times more toxic to fish than glyphosate itself.[144]

Acute toxicities of glyphosate vary widely: median lethal concentrations (LC_{50}s; the concentrations killing 50 percent of a population of test animals) from 10 ppm to over 200 ppm have been reported depending on the species of fish and test conditions.[2]

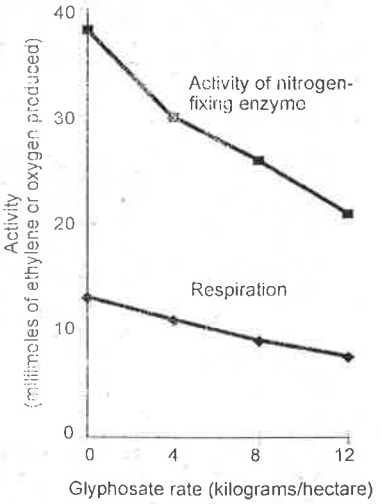
Acute toxicities (LC_{50}) of Roundup to fish range from 2 ppm to 55 ppm.[2] Part of this variability is due to age: young fish are more sensitive to Roundup than are older fish.[144] (See Figure 9.) Acute toxicities of Rodeo (used with the surfactant X-77 per label recommendations) vary from 120 to 290 ppm.[149]

In soft water there is little difference between the toxicities of glyphosate and Roundup.[150] Also, if fish have not recently eaten, the toxicity of glyphosate (LC_{50} = 2.9 ppm) is similar to that of Roundup.[151]

Roundup toxicity increases with increased water temperature. In both rainbow trout and bluegills, toxicity about doubled between 7 and 17°C (45 and 63°F).[144] Treatment of riparian areas with glyphosate causes water temperatures to increase for several years following treatment[152] because the herbicide kills shading vegetation. This means that use of glyphosate could cause increased toxicity to fish. In addition, the temperature increase could be critical for fish, like juvenile salmon, that thrive in cold water.

Sublethal effects of glyphosate occur at low concentrations. In rainbow trout and *Tilapia* concentrations of about 1/2 and 1/3 of the LC_{50} (respectively) caused erratic swimming.[153,154] The trout also

Figure 10
Effect of Glyphosate on a Nitrogen-Fixing Bacteria



Santos, A. and M. Flores. 1995. Effects of glyphosate on nitrogen fixation of free-living heterotrophic bacteria. *Lett. Appl. Microbiol.* 20:349-352.

exhibited labored breathing.[153] These effects can increase the risk that the fish will be eaten, as well as affecting feeding, migration, and reproduction.[154] Less than 1 percent of the LC_{50} caused gill damage in carp and less than 2 percent caused changes in liver structure.[155]

Birds: Glyphosate has indirect impacts on birds. Because glyphosate kills plants, its use can create a dramatic change in the structure of the plant community. This affects bird populations, since the birds depend on the plants for food, shelter, and nest support.

For example, a study of four glyphosate-treated clear-cuts (and an unsprayed control plot) in Nova Scotia found that the densities of the two most common species of birds (white-throated sparrow and common yellowthroat) decreased for two years after treatment. By the fourth year post-spray, densities had returned to normal for these two species. By then the unsprayed plot had been colonized by new species of birds (warblers, vireos, and a hummingbird) which were not found on the sprayed plots.[156]

An earlier three year study of songbird abundance following glyphosate treatment of clear-cuts in Maine forests showed similar results. Abundances of the total number of birds and three common species decreased. The decrease in bird abundance was correlated with decrease in the diversity of the habitat.[157]

Black grouse avoided glyphosate-treated clear-cuts in Norway for several years after treatment.[158] Researchers recommended that the herbicide not be used near grouse courtship areas.

Small mammals: In field studies, small mammals have been indirectly affected when glyphosate kills the vegetation they (or their prey) use for food or shelter. On clear-cuts in Maine,[141] insect-eating shrews declined for three years post-treatment; plant-eating voles declined for two. (See Figure 7.) A second study in Maine after a Roundup treatment[159] found similar results for voles. In British Columbia, deer mice populations were 83 percent lower following glyphosate treatment.[160] Another study from British Columbia found declines in chipmunk populations after Roundup treatment.[161] In Norway, there was a "strong reduction" in use of sprayed clear-cuts by mountain hare.[162] Other studies have not found impacts on small mammals,[163] suggesting that the particular characteristics of the site and the herbicide application are significant.

Wildlife: Canadian research has documented that plants serving as important food sources for wildlife are significantly damaged by glyphosate. "Severe" or "very severe damage" was recorded for 46 percent of the important food species eaten by moose, between 34 and 40 percent of the species eaten by elk, and 36 percent of the species eaten by mule deer.[164]

Effects on Nontarget Plants

As a broad-spectrum herbicide, glyphosate has potent acutely toxic effects on most plant species. There are also other kinds of serious effects. These include effects on endangered species, reduced seed quality, reduction in the ability to fix nitrogen, increased susceptibility to plant diseases, and reduction in the activity of mycorrhizal fungi.

Endangered species: Because many plants are susceptible to glyphosate, it can seriously impact endangered plant species. The U.S. Fish and Wildlife Service has identified 74 endangered plant species that it believes could be jeopardized by glyphosate. This list is based on the use of glyphosate on 9 crops, and does not include over 50 other uses.[142]

Seed Quality: Sublethal treatment of cotton with Roundup "severely affects seed germination, vigor and stand establishment under field conditions." At the lowest glyphosate rate tested, seed germination was reduced between 24 and 85 percent and seedling weight was reduced between 19 and 83 percent.[165]

Nitrogen fixation: Most living things cannot use nitrogen in its common form and instead use ammonia and nitrates, much rarer compounds. Ammonia and nitrates are created by processes called nitrogen fixation and nitrification. They are carried out by bacteria which can be found in soil and in nodules on roots of legumes and certain other plants.[166]

Studies showing effects of glyphosate on nitrogen fixation include the following: At a concentration corresponding to typical application rates, glyphosate reduced by 70 percent the number of nitrogen-fixing nodules on clover planted 120 days after treatment[167]; a similar concentration of a glyphosate herbicide reduced by 27 percent the number of nodules on hydroponically grown clover[168]; a similar concentration of glyphosate reduced by 20 percent nitrogen-fixation by a soil bacteria[169] (see Figure 10); a concentration of glyphosate approximately that expected in soybean roots following treatment inhibited the growth of soybean's nitrogen-fixing bacteria between 10 and 40 percent[170]; and treatment with a glyphosate herbicide at the lowest concentration tested (10 times typical application rates) reduced the number of nodules on clover between 68 and 95 percent.[171]

All of the studies summarized above were done in the laboratory. In the field, such effects have been difficult to observe. However, use of genetically-engineered

JOURNAL OF PESTICIDE REFORM/FALL 1998 • VOL.18, NO. 3
UPDATED 10/00

glyphosate-tolerant crop plants means that nitrogen-fixing bacteria in field situations "could be affected by repeated applications of glyphosate."[170]

Glyphosate also impacts other parts of the nitrogen cycle. A Canadian study found that treatment of a grass field with Roundup increased nitrate loss up to 7 weeks after treatment. The increase was probably caused by the nutrients released into the soil by dying vegetation.[172]

Mycorrhizal fungi: Mycorrhizal fungi are beneficial fungi that live in and around plant roots. They help plants absorb nutrients and water and can protect them from cold and drought.[173] Roundup is toxic to mycorrhizal fungi in laboratory studies. Effects on some species associated with conifers have been observed at concentrations of 1 part per million (ppm), lower than those found in soil following typical applications.[174,175] In orchids, treatment with glyphosate changed the mutually beneficial interaction between the orchid and its mycorrhizae into a parasitic interaction (one that does not benefit the plant).[176]

Plant diseases: Glyphosate treatment increases the susceptibility of crop plants to a number of diseases. For example, glyphosate increased the susceptibility of tomatoes to crown and root disease[177]; reduced the ability of bean plants to defend themselves against the disease anthracnose[178]; increased the growth of take-all disease in soil from a wheat field and decreased the proportion of soil fungi which was antagonistic to the take-all fungus[179]; and increased soil populations of two important root pathogens of peas.[180] In addition, Roundup injection of lodgepole pine inhibited the defensive response of the tree to blue stain fungus.[181]

Both the inhibition of mycorrhizae and the increased susceptibility to disease have been observed in laboratory, not field, studies. Given the serious consequences these kinds of effects could have, more research is crucial.

Plant Resistance

Plants that are resistant to glyphosate are able to tolerate treatment without showing signs of toxicity. Although many weed scientists argue that "it is nearly impossible for glyphosate resistance to evolve in weeds,"[182] others argue that "there are few constraints to weeds evolving resistance." The second group of scientists appears to be correct. In 1996 an Australian researcher reported that a population of annual ryegrass had developed resistance and tolerated five times the recommended field application rate.[183]

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Glyphosate perturbs the gut microbiota of honey bees

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Glyphosate, the primary herbicide used globally for weed control, targets the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme in the shikimate pathway found in plants and some microorganisms. Thus, glyphosate may affect bacterial symbionts of animals living near agricultural sites, including pollinators such as bees. The honey bee gut microbiota is dominated by eight bacterial species that promote weight gain and reduce pathogen susceptibility. The gene encoding EPSPS is present in almost all sequenced genomes of bee gut bacteria, indicating that they are potentially susceptible to glyphosate. We demonstrated that the relative and absolute abundances of dominant gut microbiota species are decreased in bees exposed to glyphosate at concentrations documented in the environment. Glyphosate exposure of young workers increased mortality of bees subsequently exposed to the opportunistic pathogen *Serratia marcescens*. Members of the bee gut microbiota varied in susceptibility to glyphosate, largely corresponding to whether they possessed an EPSPS of class I (sensitive to glyphosate) or class II (insensitive to glyphosate). This basis for differences in sensitivity was confirmed using in vitro experiments in which the EPSPS gene from bee gut bacteria was cloned into *Escherichia coli*. All strains of the core bee gut species, *Snodgrassella alvi*, encode a sensitive class I EPSPS, and reduction in *S. alvi* levels was a consistent experimental result. However, some *S. alvi* strains appear to possess an alternative mechanism of glyphosate resistance. Thus, exposure of bees to glyphosate can perturb their beneficial gut microbiota, potentially affecting bee health and their effectiveness as pollinators.

honey bees | microbiome | glyphosate | *Snodgrassella alvi* | *Serratia*

The broad-spectrum herbicide glyphosate [*N*-(phosphonomethyl)glycine] has long been the primary weed management system, and its use is growing in connection with crops genetically engineered to be resistant to glyphosate (1, 2). Its mechanism of action, inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), an enzyme in the shikimate pathway, prevents the biosynthesis of aromatic amino acids and other secondary metabolites in plants and some microorganisms (3). EPSPS catalyzes the reaction between phosphoenolpyruvate (PEP) and shikimate 3-phosphate (S3P) (4), and glyphosate is a competitive inhibitor that blocks the PEP-binding site (5). EPSPS enzymes from different organisms vary in molecular weight (46–178 kDa) and sequence homology (6) and form two phylogenetic clusters that differ in tolerance to glyphosate. Class I enzymes are sensitive to glyphosate and are present in all plants and in some bacteria, such as *Escherichia coli* (4); class II enzymes are only found in some bacteria, such as *Staphylococcus aureus*, and can tolerate high concentrations of glyphosate (7, 8).

Animals lack the shikimate pathway, which is why glyphosate is considered one of the least toxic pesticides used in agriculture (9). However, some evidence suggests that glyphosate affects nontarget organisms, for example, changing the behavior of honey bees (10), reducing reproduction of soil-dwelling earthworms (11), and affecting the growth of microalgae and aquatic bacteria (12). Glyphosate is also associated with changes in plant endophytic and rhizosphere microbiomes (2) and with disturbances of gut microbiota of animals living near agricultural sites (13).

Honey bees and bumble bees are major pollinators of flowering plants, including many crops. When foraging, they can be exposed to a variety of xenobiotics, such as glyphosate. This herbicide is known to affect the growth of microorganisms (13–15), and the health of bees is intrinsically related to their distinct gut microbial community (16, 17). The honey bee gut microbiota is dominated by eight bacterial species: *Lactobacillus* spp. Firm-4, *Lactobacillus* spp. Firm-5 (phylum Firmicutes), *Bifidobacterium* spp. (phylum Actinobacteria), *Snodgrassella alvi*, *Gilliamella apicola*, *Frischella perrara*, *Bartonella apis*, and Alpha 2.1 (phylum Proteobacteria) (18). Each of these species exhibits strain diversity corresponding to differences in metabolic capabilities and tolerances to xenobiotics (19, 20). Newly emerged workers (NEWs) are nearly free of gut bacteria and acquire their normal microbial community orally through social interactions with other workers during the first few days after emergence (21). Bees deprived of their normal microbiota show reduced weight gain and altered metabolism (22), increased pathogen susceptibility (17), and increased mortality within hives (23).

In this study, we investigated the effects of glyphosate exposure on the size and composition of the honey bee gut microbiome. We found the microbiome was affected by glyphosate exposure during and after gut colonization, and that glyphosate exposure during early gut colonization increased mortality of bees exposed to an opportunistic pathogen. Additionally, bee gut bacteria differ in glyphosate susceptibility. We explored the molecular mechanisms of this variability in glyphosate tolerance by expressing the EPSPS of bee gut symbionts in *E. coli*. Some bee gut bacteria tolerate glyphosate by virtue of a class II EPSPS, but a few strains with susceptible class I EPSPS depend on other,

Significance

Increased mortality of honey bee colonies has been attributed to several factors but is not fully understood. The herbicide glyphosate is expected to be innocuous to animals, including bees, because it targets an enzyme only found in plants and microorganisms. However, bees rely on a specialized gut microbiota that benefits growth and provides defense against pathogens. Most bee gut bacteria contain the enzyme targeted by glyphosate, but vary in whether they possess susceptible versions and, correspondingly, in tolerance to glyphosate. Exposing bees to glyphosate alters the bee gut community and increases susceptibility to infection by opportunistic pathogens. Understanding how glyphosate impacts bee gut symbionts and bee health will help elucidate a possible role of this chemical in colony decline.

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APPLIED BIOLOGICAL SCIENCES

yet unknown, mechanisms for tolerance. Overall, our results show that glyphosate exposure can perturb the gut microbiota of honey bees, and that compositional shifts typically favor species tolerant to glyphosate and disfavor sensitive species.

Results and Discussion

Glyphosate Perturbs the Honey Bee Gut Bacterial Community. Hundreds of adult worker bees were collected from a single hive, treated with either 5 mg/L glyphosate (G-5), 10 mg/L glyphosate (G-10) or sterile sucrose syrup (control) for 5 d, and returned to their original hive. Bees were marked on the thorax with paint to make them distinguishable in the hive. Glyphosate concentrations were chosen to mimic environmental levels, which typically range between 1.4 and 7.6 mg/L (24), and may be encountered by bees foraging at flowering weeds. To determine the effects of glyphosate on the size and composition of the gut microbiome, 15 bees were sampled from each group before reintroduction to the hive (day 0) and postreintroduction (day 3), and relative and absolute abundances of gut bacteria were assessed using deep amplicon sequencing of the V4 region of the bacterial 16S rRNA gene and quantitative PCR (qPCR).

At day 0, glyphosate exposure had little effect on the bee gut microbiome size, but the absolute and relative abundances of the core species, *S. alvi*, were significantly lower in the G-10 group (Fig. 1 and *SI Appendix*, Fig. S1). The effects of glyphosate exposure on the bee gut microbiome were more prominent at day 3, after treated bees were returned to the hive. The total number of gut bacteria decreased for both treatment groups, relative to control, but this drop was significant only for the G-5 group, which also exhibited more severe compositional shifts (Fig. 1). The absolute abundances of four dominant gut bacteria, *S. alvi*, *Bifidobacterium*, *Lactobacillus* Firm-4 and Firm-5 were decreased (Fig. 1), and the relative abundance of *G. apicola* increased in the G-5 group (*SI Appendix*, Fig. S1). Surprisingly, only *Lactobacillus* Firm-5 decreased in absolute abundance in the G-10 group (Fig. 1). This experiment was repeated using bees from a different hive and season, and similar trends were observed (*SI Appendix*, Fig. S2). As in the first experiment, significant reductions in abundance were observed for *S. alvi* in bees treated with glyphosate (*SI Appendix*, Fig. S2).

The relative lack of effects of the G-10 treatment on the microbiota composition at day 3 posttreatment is unexplained, but may reflect other effects of glyphosate on bees. Our recapture method fails to sample bees that died or abandoned the hive. Since bees exposed to glyphosate may exhibit impaired spatial processing, compromising their return to hives (10, 24), bees in the G-10 group that consumed more glyphosate-laced sugar syrup before reintroduction to the hive may have been less likely to return to the hive after foraging. Since fewer than 20% of bees

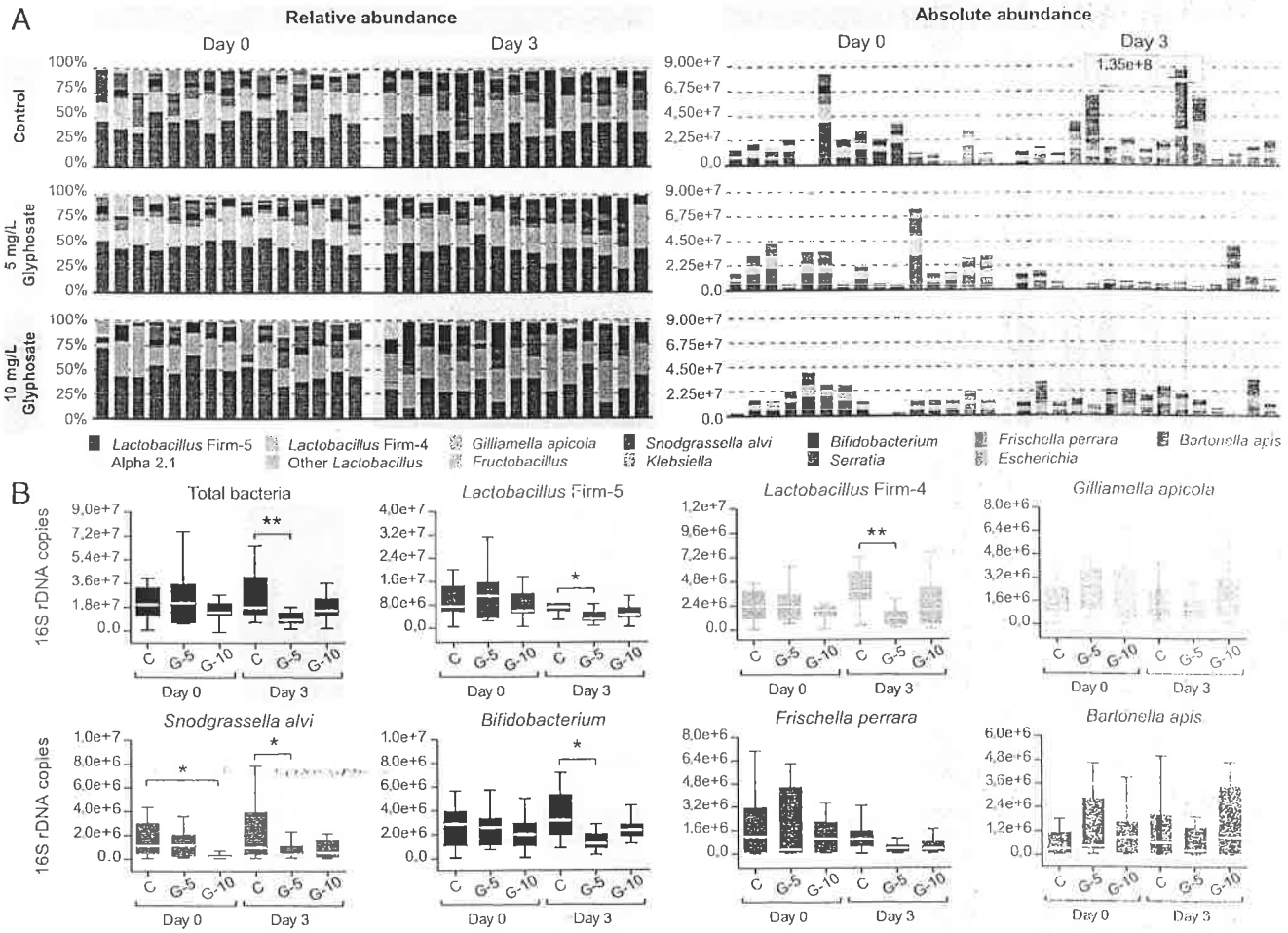


Fig. 1. Changes in gut microbiota composition following glyphosate exposure of honey bees with established gut communities. (*A*) Stacked column graph showing the relative and absolute abundances of gut bacterial species in control bees and bees treated with 5 mg/L or 10 mg/L glyphosate at posttreatment days 0 and 3. Each column represents one bee. (*B*) Boxplots of bacterial 16S rDNA copies for control (C) and glyphosate-treated (G-5 and G-10) bees at posttreatment days 0 and 3 ($n = 15$ for each group and time point). Box-and-whisker plots show high, low, and median values, with lower and upper edges of each box denoting first and third quartiles, respectively. $^*P < 0.05$ and $^{**}P < 0.01$, Wilcoxon rank sum test followed by Bonferroni correction.

reintroduced to the hive were recovered, recovered bees may not represent the total effect of glyphosate on treatment groups.

Glyphosate Affects Early Gut Bacterial Colonization. Glyphosate arrests bacterial growth without directly killing the cells, so we hypothesized that it would have a greater effect on actively dividing cells present during early gut colonization. To test this, NEWs, which are nearly free of gut bacteria (21), were simultaneously exposed to an inoculum consisting of their normal microbial community and to glyphosate. This simultaneous exposure is relevant to field situations, since glyphosate has been detected in hives and honey samples (25, 26), indicating that honey bee foragers can transport residues of this herbicide to the colony and contaminate other bees, including NEWs, and food resources. Also, glyphosate is a stable, water-soluble chemical that can persist in the environment for long periods (10).

Assessment of gut microbiomes, as described in the previous section, identified all eight core gut taxa in both control and treatment groups (Fig. 2*A*), showing that glyphosate does not eliminate colonization by any core member. Average total bacterial abundance was slightly lower in glyphosate-treated bees, but this was not statistically significant (Fig. 2*B*). *S. alvi* was the most strongly affected member of the gut microbiota and decreased in both absolute and relative abundance, while *Lactobacillus* Firm-4 increased in relative abundance (Fig. 2 *C–E* and *SI Appendix*, Fig. S3). Based on relative abundance, gut community compositions of glyphosate-treated bees differed from those of controls (principal coordinate analysis of weighted UniFrac) (27), permanova test with 9,999 permuations; $P = 0.0078$, pseudo-F statistic = 6.66) (Fig. 2*F*). Thus, glyphosate exposure during early development of the gut community can interfere with normal colonization by altering the abundance of beneficial bacterial species.

Typically, captive honey bees do not defecate, and dead bacterial cells and the released DNA accumulate in the gut (23). Thus, we also analyzed changes in bacterial abundance after glyphosate exposure by extracting both DNA and RNA from the guts of treatment and control bees in a second colonization experiment. We included a positive control group, in which bees were exposed to tylosin, an antibiotic used in beekeeping. This antibiotic treatment was expected to perturb the microbiota, but the decrease was significant only for RNA samples (*SI Appendix*, Fig. S4). Glyphosate exposure resulted in nonsignificant decreases in total bacteria for both DNA and RNA assays. We also checked changes in absolute abundance for three core bacterial species, *S. alvi*, *Lactobacillus* Firm-4, and *Lactobacillus* Firm-5. Tylosin treatment resulted in reductions for 16S rRNA copies (*SI Appendix*, Fig. S4). Effects of glyphosate treatment were specific to *S. alvi*, which was the only assayed species showing significant reductions in absolute abundance, observed for both DNA and RNA assays (*SI Appendix*, Fig. S4). This experiment suggests that measures based on DNA are partly obscured by DNA from dead bacterial cells, although this effect does not entirely mask shifts in bacterial abundance.

Glyphosate Exposure Makes Young Worker Bees More Susceptible to *Serratia*. To determine whether glyphosate-induced perturbation of microbiota colonization affects host health, we measured the susceptibility of glyphosate-treated bees to an opportunistic bacterial pathogen. NEWs were exposed to glyphosate in the stage of acquiring their normal microbial community. After 5 d of treatment, bees were challenged with *Serratia marcescens* kz19, an opportunistic pathogen commonly detected at very low frequencies in the bee gut (28, 29).

For bees lacking gut microbiota, *Serratia* challenge resulted in increased mortality relative to that observed for bees with a conventional gut microbiota, regardless of glyphosate exposure (Fig. 2*G* and *SI Appendix*, Fig. S5). For bees with a conventional gut microbiota, glyphosate treatment resulted in increased mortality after *Serratia* challenge. To determine whether this increased mortality was attributable to the effects of glyphosate on the gut microbiota or to direct effects of glyphosate on bees, we included

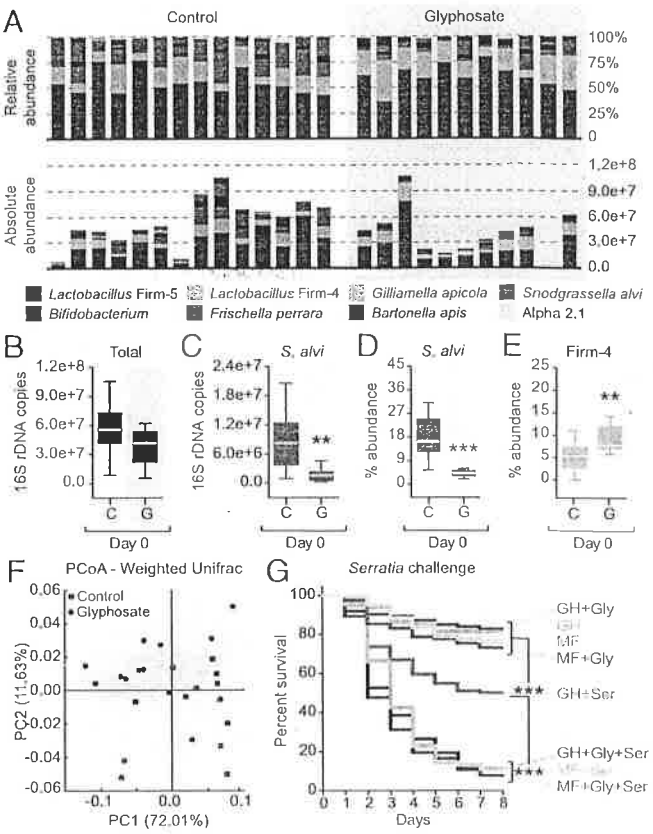


Fig. 2. Changes in gut microbiota composition following glyphosate exposure of young honey bees and susceptibility to *Serratia* infection. (*A*) Stacked column graph showing the relative and absolute abundances of gut bacterial species in control and glyphosate-treated bees. Each column represents one bee. (*B–E*) Boxplots of total bacterial 16S rDNA copies and of absolute and relative abundances of two gut bacterial species for control ($n = 14$) and glyphosate-treated ($n = 11$) bees. $^{**}P < 0.01$, and $^{***}P < 0.001$, Wilcoxon rank sum test followed by Bonferroni correction. (*F*) Principal coordinate analysis of gut community composition using weighted UniFrac (permanova test with 9,999 permuations; $P = 0.0078$, pseudo-F statistic = 6.66). (*G*) The percent survival of age-controlled bees after *Serratia* kz19 exposure, shown as a Kaplan–Meier survival curve. $^{***}P < 0.001$, coxph model implemented in the "survival" package in R. GH, gut homogenate-exposed bees; Gly, glyphosate treatment; MF, microbiota-free bees; Ser, *Serratia* challenge.

control groups not challenged with *Serratia*. In bees exposed to glyphosate, but not challenged with *Serratia*, survival rates were not significantly affected by glyphosate and were much higher than in the *Serratia*-challenged groups (Fig. 2*G*), demonstrating that a direct effect of glyphosate on bees is not the basis of the high mortality of glyphosate-exposed, pathogen-challenged bees.

Our results show that glyphosate reduces the protective effect of the gut microbiota against opportunistic pathogens and that *S. alvi* is the bacterial species most negatively affected by glyphosate exposure. By itself, *S. alvi* appears to give some protection, but not as much as the whole gut microbiota (*SI Appendix*, Fig. S6). *S. alvi* forms a biofim on the wall of the gut ileum (18, 21, 30), which may function as a mechanical barrier against pathogen invasion. Some *S. alvi* strains encode type VI secretion systems (31), which could contribute to colonization resistance through contact-dependent inhibition of *Serratia*. Furthermore, host expression of antimicrobial peptides is upregulated after *S. alvi* colonization (32), which could increase resistance to infection by pathogens. Besides a direct protective effect, *S. alvi* may be critical in enabling the full microbiota to assemble, thus enabling greater protection.

The Bee Gut Contains Bacterial Species with both Sensitive and Insensitive Types of EPSPS. Bacterial EPSPS exists as two main types, corresponding to two phylogenetic clusters, that differ in sensitivity to glyphosate: Class I is naturally sensitive, whereas class II is insensitive (8). To identify the EPSPS types present in the bee gut microbiota, a phylogenetic tree was constructed using the EPSPS protein of bacterial strains isolated from honey bee and bumble bee guts and of other representative organisms (Fig. 3). EPSPS sequences from *S. alvi*, *G. apicola*, *F. perrara*, *Bifidobacterium*, and *Apibacter adventoris* (phylum Bacteroidetes) (33) clustered with those from other organisms containing a class I EPSPS, and thus these bacteria are predicted to be sensitive to glyphosate. In contrast, sequences from *B. apis* and *Lactobacillus* Firm-4 clustered with other bacteria containing a class II EPSPS, as did *Parasaccharibacter apium* (Alpha 2.2), a bacterium commonly detected in honey bee larvae and hives, but rare in the guts of workers (34), and *Paenibacillus larvae*, the agent of American foulbrood in honey bee larvae (35); these bacteria are predicted to be unaffected by glyphosate exposure. *Lactobacillus* Firm-5 strains for which genomes are available lack the gene encoding EPSPS and were excluded from our analysis.

Bee Gut Bacteria Vary in Glyphosate Sensitivity at the Species and Strain Levels. Several bee gut-associated bacterial strains isolated from honey bees and bumble bees were cultured in vitro in the presence or absence of a high dose of glyphosate. Most *S. alvi* and *G. apicola* strains tested, which contain a class I EPSPS, either do not grow or have a delay in growth when cultured in the presence of glyphosate; no such effect is observed for strains containing a class II EPSPS, *Lactobacillus* Firm-4 and *B. apis* (Fig. 3 and *SI Appendix*, Fig. S7). However, *S. alvi* strains wkB2 and wkB298, despite containing a class I EPSPS, grow as well in the presence of glyphosate as in its absence, with no initial delay in growth. We looked for potential single-site mutations in the EPSPS active site of these strains, which is known to confer tolerance to glyphosate (36), but no mutations were observed, indicating that the resistance in these *S. alvi* strains results from other mechanisms.

A previous study of the genes required by *S. alvi* to live in the bee hindgut showed that the aromatic amino acid biosynthetic pathway is required for growth in this niche (30), which is consistent with low aromatic amino acid concentrations in the hindgut (37). Thus, bee gut bacterial strains having a glyphosate-susceptible EPSPS are predicted to drop in abundance following exposure, as observed for *S. alvi* (Fig. 1 and *SI Appendix*, Fig. S2) and *Bifidobacterium* (Fig. 1) in the hive experiments. *Lactobacillus* Firm-4, which encodes a class II EPSPS, and Firm-5, which does not contain the target enzyme of glyphosate, also had their abundances reduced in the hive experiment (Fig. 1), which was not expected. This may be explained by the fact that these strains lack the aromatic amino acid biosynthetic pathway (18), relying on uptake of aromatic amino acids released by other bacterial species, such as *S. alvi*, in the bee gut environment. The increase in *G. apicola* relative abundance (*SI Appendix*, Fig. S1) was unpredicted, but was also observed in a previous study on microbial community responses to antibiotic perturbation (23).

Glyphosate Resistance Is Independent of EPSPS Class in Some Bee Gut Strains. To understand the mechanism that prevents some bee gut bacterial strains from growing in the presence of glyphosate, we complemented *E. coli* Δ*aroA* with *aroA* genes cloned from bee gut bacterial strains as well as with the *E. coli* K12 *aroA*, which is known to be sensitive to glyphosate. *E. coli* Δ*aroA* cannot synthesize aromatic amino acids and does not grow in minimal media, but grows normally when transformed with an arabinose-inducible plasmid carrying the intact *E. coli aroA* gene (Fig. 4).

Transformants carrying the *aroA* gene from *S. alvi*, *G. apicola*, and *B. apis* were able to grow in minimal media at a similar rate to the transformant carrying the *aroA* gene from *E. coli* (Fig. 4). The addition of 10 mM glyphosate to the media resulted in a delay in growth of ~48–72 h for transformants carrying the *aroA* gene from

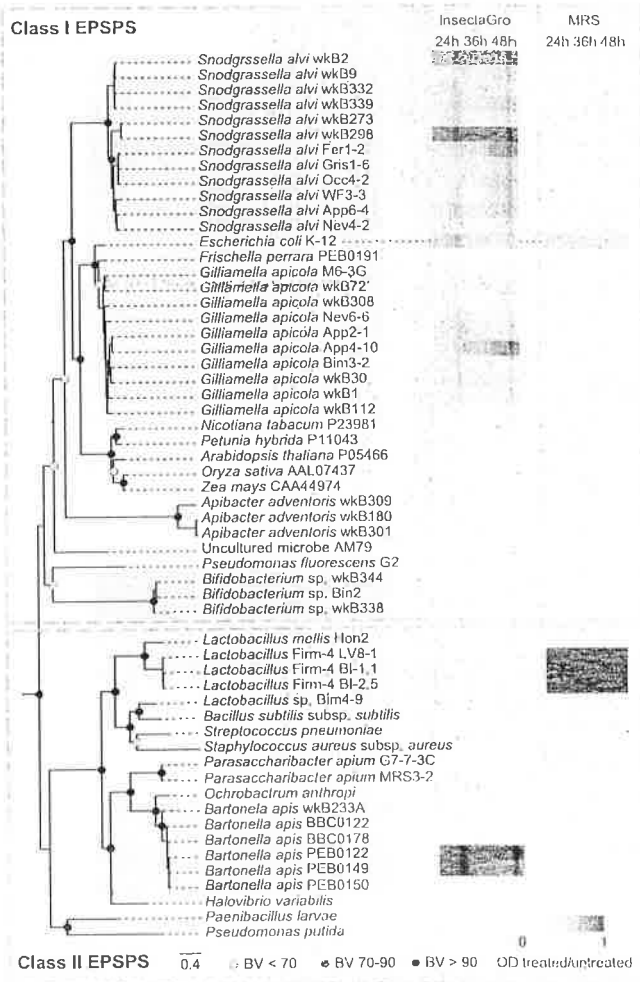


Fig. 3. Maximum-likelihood phylogeny based on amino acid sequences of EPSPS (PhyML 3.1, LG model + Gamma4, 100 bootstrap replicates). Bee gut bacterial strains, other bacteria, and some plant species are represented in the phylogeny. The heatmap represents the growth of some bee bacterial strains in the presence/absence of 10 mM glyphosate at three time points (24, 36, and 48 h). Glyphosate was dissolved in the culture media (InsectaGro or MRS broth, based on bacterial preferences). A value of 1 indicates that growth is the same in the presence or absence of glyphosate.

all *S. alvi* and *G. apicola* strains tested (Fig. 4). This is expected if glyphosate binds to a susceptible EPSPS, blocking the shikimate pathway and preventing bacterial growth until the concentration of PEP or EPSPS exceeds that of glyphosate, allowing the transformants to resume growth. On the other hand, the transformant carrying the *aroA* gene from *B. apis* did not exhibit the growth delay in the presence of glyphosate (Fig. 4), as predicted since this *aroA* version encodes an insensitive class II EPSPS. Moreover, the addition of increased concentrations of arabinose in the media or reduction in glyphosate concentration sped up the growth of all transformant strains (*SI Appendix*, Fig. S8), which corroborates the reversible mechanism of EPSPS inhibition by glyphosate.

Although *S. alvi* strains wkB2 and wkB298 were resistant to glyphosate (Fig. 3 and *SI Appendix*, Fig. S7), their *aroA* versions were sensitive to glyphosate (Fig. 4). Potentially, some bee gut microbes may have evolved alternative glyphosate resistance mechanisms due to a history of exposure, similar to the resistance observed for the antibiotic tetracycline used in beekeeping (38). Therefore, we overexpressed, in WT *E. coli*, certain genes encoding transporters from some bee gut bacteria, including wkB2 and wkB298 strains, that could be involved in

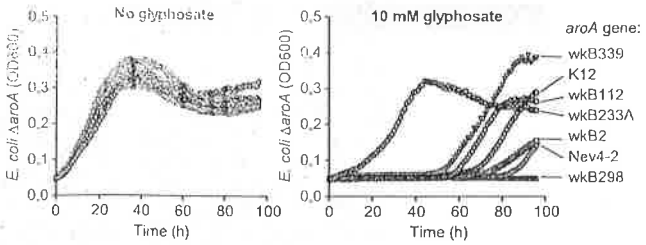


Fig. 4. Growth curves of *E. coli* Δ*aroA* BW25113 expressing the *aroA* gene from different bee-associated bacterial strains (*B. apis* in red, *E. coli* in black, *G. apicola* in blue, and *S. alvi* in green) cultured in minimal media in the presence or absence of 10 mM glyphosate.

glyphosate resistance: *yhhS*, which encodes a membrane transporter conferring glyphosate tolerance when overexpressed in *E. coli* (39), and *tetC*, which encodes an efflux pump that provides tetracycline resistance to *S. alvi* wkB2 (38). However, these transporters were not able to reverse the delay in *E. coli* growth caused by glyphosate (*SI Appendix*, Fig. S9). As the glyphosate tolerance exhibited by some *S. alvi* strains does not appear to be due to a resistant EPSPS or to transport by YhhS or TetC, these strains are likely to employ a novel mechanism of glyphosate resistance. Future studies might identify this mechanism and determine the evolutionary origin of resistance.

S. alvi Strains May Vary in Sensitivity to Glyphosate in Vivo. Our phylogenetic analysis and cloning experiments demonstrated that, despite displaying variable susceptibility to glyphosate in vitro, all *S. alvi* strains possess a glyphosate-sensitive class I EPSPS. Therefore, we investigated whether this variation in susceptibility occurs in vivo. NEWs were monoinoculated with two different *S. alvi* strains: wkB2, which grows in the presence of high concentrations of glyphosate, or wkB339, which exhibits a delay in growth in the presence of high concentrations of glyphosate (Fig. 5*A*). Bees were hand fed bacterial suspensions to inoculate with a control number of *S. alvi* cells, exposed to glyphosate for 3 d, and sampled at days 1 and 3 during exposure. Both *S. alvi* wkB2 and *S. alvi* wkB339 increased in abundance between days 1 and 3 in unexposed bees, consistent with previous findings that *S. alvi* can colonize guts of monoinoculated bees (30, 40). Based on qPCR estimates of *S. alvi* abundance on day 3, glyphosate exposure had a negative effect on growth of both strains [two-way analysis of variance (ANOVA), treatment effect, $P < 0.0001$]. *S. alvi* wkB339 was more affected by glyphosate exposure, based on significant interaction between strain and treatment (two-way ANOVA, $P < 0.0204$). Correspondingly, the absolute abundance of the glyphosate-sensitive strain, wkB339, was significantly lower in glyphosate-treated bees compared with controls (Tukey's test, $P < 0.001$) or wkB2-treated bees (Tukey's test, $P < 0.001$) (Fig. 5 *B* and *C*). Potentially, strain differences in glyphosate sensitivity may contribute to the observed variation in the overall decrease in *S. alvi* abundance when bees with their native gut microbiota are exposed to glyphosate.

Conclusion

As in many animals, honey bees rely on their gut microbial community for a variety of functions, including food processing (25, 26), regulation of immune system (33, 34), and defense against pathogens (17, 27). Perturbations of this system have the potential to lead to negative consequences for host fitness. We found that glyphosate affects the bee gut microbiota composition and that bacterial species and strains within this community vary in susceptibility to glyphosate. Recent experimental and observational studies have provided evidence that dysbiosis affecting the bee gut can increase susceptibility to pathogen invasion (23, 41, 42). Our results also suggest that establishment of a normal microbial community is crucial for protection against opportunistic pathogens of honey bees. Furthermore, our results highlight one potential mechanism by which glyphosate affects bee health.

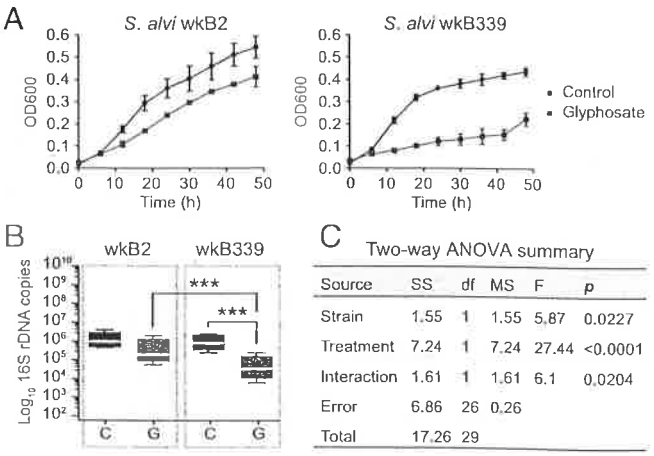
While some species in the bee gut can tolerate high concentrations of glyphosate due to the presence of a class II EPSPS enzyme, others are sensitive due to the presence of a class I EPSPS. A consistent effect of glyphosate on the bee gut microbiota was a negative impact on growth of *S. alvi*, which possesses a sensitive EPSPS. However, some strains of *S. alvi* may tolerate glyphosate through an as yet unknown mechanism. Since bee gut symbionts affect bee development, nutrition, and defense against natural enemies, perturbations of these gut communities may be a factor making bees more susceptible to environmental stressors including poor nutrition and pathogens.

Methods

More details are provided in *SI Appendix*.

Effects of Glyphosate on the Honey Bee Gut Microbiome. Adult workers with established gut communities were collected from a hive at University of Texas, Austin (UT Austin), marked on the thorax with paint, fed glyphosate (5 or 10 mg/L) or sterile sucrose syrup for 5 d, and returned to the same hive. Fifteen bees from each group were sampled before and 3 d after reintroduction to the hive. This experiment was repeated using bees from a different hive and different year. DNA was extracted from dissected guts and used as template for qPCR analyses. DNA samples from the first experiment were submitted for Illumina sequencing at the Genomic Sequencing and Analysis Facility (GSAF) at UT Austin. Illumina sequence reads were processed using QIIME 1.9.1 (43).

Effects of Glyphosate on Early Gut Colonization and Susceptibility to *Serratia* Infection. Hundreds of late-stage pupae were removed from brood frames and allowed to emerge under sterile conditions in laboratory. (Experiment A) NEWs were exposed to bee gut homogenate for 5 d, then hand fed 1 mM glyphosate or sterile sugar syrup on 2 alternate days. Fifteen bees from each group were sampled 2 d after the last hand feeding. DNA was extracted from dissected guts, used as template for qPCR analyses, and submitted for Illumina sequencing at the GSAF, UT Austin. (Experiment B) NEWs were exposed to a bee gut homogenate or sterile sucrose syrup. Each group was divided into two subgroups and treated with 0.1 mM glyphosate or sterile sucrose syrup for 5 d. After that, half of the subgroups was exposed to the opportunistic pathogen *S. marcescens* kz19, whereas the other half was used as controls.



C Two-way ANOVA summary

Source	SS	df	MS	F	p
Strain	1.55	1	1.55	5.87	0.0227
Treatment	7.24	1	7.24	27.44	<0.0001
Interaction	1.61	1	1.61	6.1	0.0204
Error	6.86	26	0.26		
Total	17.26	29			

Fig. 5. Variation in *S. alvi* strain sensitivity to glyphosate. (*A*) Growth curves of *S. alvi* wkB2 and wkB339 cultured in InsectaGro media in the presence or absence of 10 mM glyphosate. Experiment was performed in triplicate, and each data point represents the average optical density (600 nm, with SD bars). (*B*) Boxplots of *S. alvi* wkB2 and wkB339 abundances in bees exposed or not to 0.1 mM glyphosate for 3 d estimated by qPCR. Box-and-whisker plots show high, low, and median values, with lower and upper edges of each box denoting first and third quartiles, respectively. ***$P < 0.001$, two-way ANOVA with Tukey's correction for multiple comparisons. (*C*) Two-way ANOVA for effects of *S. alvi* strain and glyphosate treatment.

APPLIED BIOLOGICAL SCIENCES

Bees were exposed to similar amounts of glyphosate (~1.7 μg) in experiments A and B.

S. alvi Colonization During Glyphosate Exposure. NEWs were hand fed 5 μL sucrose syrup containing ~10^5 cells of *S. alvi* wkB2 or wkB339 or sterile sucrose syrup as control. Each group was divided into two subgroups and treated with 0.1 mM glyphosate or sterile sucrose syrup for 3 d immediately following bacterial exposure. Eight bees were sampled from each subgroup at days 1 and 3, and DNA was extracted from dissected guts. *S. alvi*-specific primers (44) were used to amplify total copies of 16S rDNA of each sample by qPCR.

In Vitro Experiments with Bee Gut Bacterial Strains. Honey bee and bumble bee gut bacterial strains (*SI Appendix*, Table S1) were cultured in InsectaGro or MRS broth in the presence or absence of 10 mM glyphosate in a 96-well plate and incubated in a plate reader at 35 °C and 5% CO_2 for 48 h. Optical density was measured at 600 nm every 6 h. Experiments were performed in triplicate.

Plasmid Construction and Transformation. The *aroA*, *yhhS*, and *tetC* genes from various bacterial strains were PCR amplified and cloned into the arabinose-inducible pBAD30 vector (45) by Gibson assembly (46) and then used to transform *E. coli* strain BW25113 or a derivative lacking the *aroA* gene by electroporation. Primer sequences are listed in *SI Appendix*, Table S2.

Growth Rate Analysis of Transformed *E. coli*. Transformed *E. coli* cells were cultured in duplicate in 24-well plates containing M9 minimal medium (47) with appropriate antibiotics, varying concentrations of glyphosate, and varying concentrations of arabinose. The plates were incubated in a plate reader at 37 °C for 24–96 h. Optical density was measured at 600 nm every hour.

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RESEARCH ARTICLE

Effects of field-realistic doses of glyphosate on honeybee appetitive behaviour

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ABSTRACT

Glyphosate (GLY) is a broad-spectrum herbicide used for weed control. The sub-lethal impact of GLY on non-target organisms such as insect pollinators has not yet been evaluated. *Apis mellifera* is the main pollinator in agricultural environments and is a well-known model for behavioural research. Honeybees are also accurate biosensors of environmental pollutants and their appetitive behavioural response is a suitable tool with which to test sub-lethal effects of agrochemicals. We studied the effects of field-realistic doses of GLY on honeybees exposed chronically or acutely to the herbicide. We focused on sucrose sensitivity, elemental and non-elemental associative olfactory conditioning of the proboscis extension response (PER), and foraging-related behaviour. We found a reduced sensitivity to sucrose and learning performance for the groups chronically exposed to GLY concentrations within the range of recommended doses. When olfactory PER conditioning was performed with sucrose reward with the same GLY concentrations (acute exposure), elemental learning and short-term memory retention decreased significantly compared with controls. Non-elemental associative learning was also impaired by an acute exposure to GLY traces. Altogether, these results imply that GLY at concentrations found in agro-ecosystems as a result of standard spraying can reduce sensitivity to nectar reward and impair associative learning in honeybees. However, no effect on foraging-related behaviour was found. Therefore, we speculate that successful forager bees could become a source of constant inflow of nectar with GLY traces that could then be distributed among nestmates, stored in the hive and have long-term negative consequences on colony performance.

KEY WORDS: *Apis mellifera*, Glyphosate, Sub-lethal effects, Associative learning, Sensitivity to reward

INTRODUCTION

Glyphosate (GLY), *N*-(phosphonomethyl) glycine, is a broad-spectrum herbicide applied for weed control (Goldsborough and Brown, 1988). In the last few decades, its consumption has increased sharply and it has become one of the most used agrochemicals worldwide (Zhang et al., 2011). Because of the upscale in monocultures and genetically modified crops, aerial application of GLY has become the most common application method and has thus widened its spread area (Giesy et al., 2000). This and other methods of application generate spray drift, which carries the herbicide away from the limits of the field cultivated with the target crop. Therefore, its widespread presence in agricultural ecosystems and their surroundings has inevitably made us wonder what effects, if any, it has on non-target organisms.

Although GLY inhibits aromatic amino acid pathways present only in plants, microorganisms and fungi (not in animals) (Amrhein et al., 1980; Carlisle and Trevors, 1988; Duke et al., 1989), there are studies that have found different negative effects in invertebrate and vertebrate species. For instance, common application concentrations have been found to cause growth deficit in the earthworm *Aporrectodea caliginosa* (Springett and Gray, 1992), and concentrations higher than 10 mg l^{-1} have been proven to have an effect on body growth in the freshwater snail *Pseudosuccinea columella* (Tate et al., 1997). In vertebrates, studies indicate that chronic exposure to different formulates with GLY concentrations ranging between 3.8 and 18 mg acid equivalents l^{-1} (mg a.e. l^{-1}) may negatively affect amphibians (Howe et al., 2004; Relyea, 2005a; Relyea, 2005b).

Honeybees, *Apis mellifera* L., are the main pollinators in agricultural ecosystems (Aizen et al., 2009). Each foraging honeybee makes trips several times a day to gather resources from several kilometres away and, in doing so, takes any foreign substances present in those resources back to the hive. Because honeybee foragers take back to the hive substances present in the resources they gather (von Frisch, 1967), agrochemicals with a high solubility in water such as GLY, which might be present in the flowers visited after a spray application (Bohan et al., 2005), may also be present in the stored honey. Substances that are taken into the hive can remain stored for long periods of time and accumulate until the resources are used as supplies for the colony (Devillers and Pham-Delègue, 2002). Hence, agrochemicals accumulated inside the hive could have subtle negative effects, often inconspicuous within the short term (Giesy et al., 2000), that could impair behavioural processes in the long term (Kirchner, 1999). As a result, honeybees are very sensitive biosensors of changes in the environment and respond even to subtle variations caused by pollutants (Devillers and Pham-Delègue, 2002). Sub-lethal effects of agrochemicals can be evaluated on honeybees through standardized laboratory assays based on appetitive behavioural responses, learning abilities, and foraging and communication skills.

Honeybee foragers can obtain information and retain a variety of cues from the environment by perceiving different sensory stimuli and establishing associations between them (Menzel, 1999). In this way, bees can learn to associate a specific odour with a reward (elemental learning) or even that an odour predicts reward only when it is part of a complex blend [e.g. non-elemental learning (Deisig et al., 2001; Giurfa, 2003; Giurfa, 2007)]. Acquisition of olfactory information has been shown to be well retained even when it occurs at young ages of the adult stage (Arenas and Farina, 2008; Arenas et al., 2009a; Arenas et al., 2012). Young workers that remain inside the hive can learn which odours are rewarded when fed with recently collected resources (Nixon and Ribbands, 1952;

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Grüter et al., 2006) or with food stored in the hive (Winston, 1987). Moreover, experiences acquired inside the colony can increase the efficiency of a colony's foraging-related tasks (Arenas et al., 2009b; Balbuena et al., 2012a). These learning abilities can be evaluated under laboratory experimental conditions through the proboscis extension response (PER). Bees extend their proboscis after their antennae have been stimulated with sucrose solution and this response can be conditioned if a neutral stimulus (e.g. an odour or another sensory stimulus) is paired with the reward (Kuwabara, 1957; Takeda, 1961; Bitterman et al., 1983; Matsumoto et al., 2012).

The PER can also be used to measure reward sensitivity. Reward sensitivity is intimately bound to associative learning (Scheiner et al., 1999; Page and Erber, 2002) and is therefore inseparable from foraging behaviours (Page et al., 1998). Changes in food source profitability found by foragers affect their threshold for appetitive responses to the extent that they modify a series of stereotyped movements used to convey information, known as the waggle dance (von Frisch, 1967). The dancers' manoeuvres encode information about the location and profitability of the discovered food source that is transmitted to the rest of the colony during the dance (von Frisch and Lindauer, 1955; Riley et al., 2005; Thom et al., 2007; Grüter and Farina, 2009a; Grüter and Farina, 2009b). This complex behavioural repertoire and the specialized skill set of workers are highly relevant and fine-tuned for colony survival and susceptible to sub-lethal effects of noxious substances.

GLY toxicity tests on *A. mellifera* for product approval did not consider sub-lethal nor prolonged exposure effects. Studies were only focused on obtaining LD_{50} (lethal dose, 50%) as a measure of the effect of an acute exposure, but nevertheless, they were carried out on the basis that honeybees might in fact be exposed to GLY in their natural environment, either through the consumption of contaminated resources or through a direct exposure as a result of inadvertent spraying (Giesy et al., 2000). Even though LD_{50} results seem to indicate that GLY is not harmful for honeybees, the fact that honeybees are potentially exposed to GLY motivated us to pursue further analysis and to address the lack of chronic studies.

We were specifically interested in the possible sub-lethal effects of GLY on *A. mellifera*. To evaluate these effects, we used GLY concentrations within a range of 0 to 3.7 mg a.e. l^{-1}, which do not exceed those recommended for aquatic and terrestrial weed control or those measured in natural environments, which are found within a 1.4 to 7.6 mg a.e. l^{-1} range (Goldsborough and Brown, 1988; Feng et al., 1990; Giesy et al., 2000). We focused on reward sensitivity (sensitivity to sucrose) and learning abilities of honeybees, processes that involve appetitive behaviours. First, we evaluated the effect of prolonged exposures to GLY at pre-foraging ages (laboratory-reared bees) on sensitivity to sucrose and on associative learning. We then studied the effect of acute exposures to GLY at foraging ages (hive-reared bees) on elemental and non-elemental associative learning and on foraging behaviour.

RESULTS

Effect of prolonged exposures to glyphosate on laboratory-reared bees

Survival, food ingestion and locomotive activity

We first investigated the effect of a prolonged exposure to GLY on the behaviour of laboratory-reared bees. Table 1 shows the results obtained for survival, ingestion and locomotive activity measured at 15 days of age on bees exposed to different GLY concentrations during the first 15 days of adult life. Although bees exposed to GLY showed a higher level of mortality than untreated bees, we found no significant differences between the three groups (one-way ANOVA: $F_{2,12}=3.67$, $P=0.057$; Table 1). This result, together with the fact that the highest accumulated mortality recorded during 15 days only reached 24%, led us to regard the GLY doses used as sub-lethal.

Before evaluating the effect of a prolonged exposure to GLY on sensitivity to sucrose and learning abilities, we studied whether it had an effect on the overall behaviour of 15-day-old bees. Food intake, mortality, mortality due to harnessing, and locomotive and orientation activity did not vary between bees exposed to different GLY concentrations (food intake: one-way ANOVA, $F_{2,12}=1.32$, $P=0.305$; survival between harnessing and PER conditioning: G-test, $G_H=0.76$, $P=0.683$, $N=579$, d.f.=2; locomotive activity: three-way RM-ANOVA, main effect GLY concentration: $F_{2,9}=0.07$, $P=0.936$, GLY concentration × LED colour interaction: $F_{2,9}=0.85$, $P=0.493$; for details, see Table 1). These results show that all bees, independently of the GLY concentration to which they were exposed, presented similar behavioural responses and survival rates at 15 days of age.

Sensitivity to sucrose

With the general behavioural results in mind, we investigated whether sensitivity to sucrose and learning performance were also intact. We first tested the sensitivity to sucrose of bees through a PER and gustatory response score protocol (PER–GRS protocol). GRS scores of bees exposed to GLY were lower than those of non-exposed bees (Kruskal–Wallis test: $H=9.54$, $P=0.007$, $N=203$, d.f.=2; Fig. 1A). This indicates that 15-day-old bees that were reared with sub-lethal concentrations of GLY present an increased response threshold for sucrose.

Table 1. Survival and behavioural variables after a prolonged exposure to glyphosate (GLY)

	GLY concentration (mg l^{-1})					
Survival and behavioural variable	0	2.5	5	Test statistic	N	P
Accumulated mortality up to day 14 per cage (%)[a]	10.3±3.7	24.1±3.7	20.1±3.7	$F_{2,12}=3.67$	5	0.057
Accumulated intake up to day 14 per cage (ml bee^{-1})[a]	0.28±0.04	0.33±0.04	0.36±0.04	$F_{2,12}=1.32$	5	0.305
Survival between harnessing and conditioning protocol (%)[b]	86	92.8	93.8	$G_H=0.76$ (d.f.=2)	193	0.685
Locomotive activity: log_{10} time between same colour lights (s)[c]						
Yellow–yellow	8.5±0.8	11.0±2.4	14.8±3.5	$F_{2,9}=0.07$	28	0.936
Green–green	14.4±3.2	10.7±1.3	12.8±2.8			

[a]One-way ANOVA.
[b]Homogeneity test (G-test).
[c]Three-way RM-ANOVA.
Caged bees were exposed to different GLY concentrations (0, 2.5 and 5 mg GLY per litre of sucrose solution) during the first 15 days of adult life. Locomotive activity was measured for two pairs of LED lights: yellow–yellow and green–green. All values are expressed as means ± s.e.m., with the exception of those corresponding to survival between harnessing and the conditioning protocol.

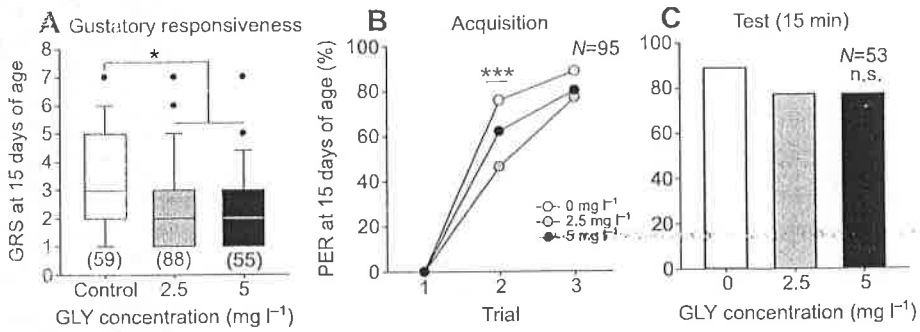

Fig. 1. Effect of a prolonged exposure to glyphosate (GLY) on sensitivity to sucrose and learning performance in honeybees. Caged bees were exposed to different GLY concentrations (0, 2.5 and 5 mg GLY per litre of 1.8 mol l^{-1} sucrose solution) during the first 15 days of their adult life. Behavioural parameters of bees at 15 days of age were tested through: (A) sensitivity to reward that was evaluated with a gustatory response score (GRS) test; (B) an absolute classical conditioning protocol in which the proboscis extension response (PER; %) towards the trained odour was quantified over the course of three acquisition trials; and (C) the conditioned response (PER) towards the trained odour alone measured 15 min after acquisition. The number of bees tested is shown in brackets below each box (A) or in the top right corner (B,C). Boxes indicate the inter-quartile range, horizontal lines within boxes indicate the medians, whiskers include all points within 1.5 times the inter-quartiles, solid circles indicate outliers [(A) Dunn comparisons: *P<0.05; (B) Tukey *post hoc* comparisons: *P<0.05; ***significant differences between treatments in the second trial].

Olfactory PER conditioning

Next, we assayed bee performance in an absolute olfactory classical conditioning protocol of the PER. Fig. 1B shows the %PER towards the conditioned stimulus [CS: linalool (LIO)] for bees of 15 days of age for the course of three acquisition trials in which the reward did not contain GLY. Bees that were exposed to sub-lethal concentrations of GLY during the first 15 days of adult life showed a lower performance than non-exposed bees. We performed a two-way repeated-measures ANOVA (RM-ANOVA) and found a significant interaction between factors (main effect GLY concentration $F_{2,282}$=7.76, P<0.001; interaction GLY concentration × acquisition trial: $F_{2,4}$=5.14, P<0.001; Fig. 1B). We therefore computed simple effects for GLY concentration and found statistical differences for GLY concentration effects for the second acquisition trial (one-way ANOVA: $F_{2,282}$=9.19, P<0.001). Tukey *post hoc* comparison tests revealed that the effects of the three GLY concentrations on the second acquisition trial differ (P<0.05). These results show that a prolonged exposure to sub-lethal concentrations of GLY during the first 15 days of adult life hinders the acquisition dynamics of the ability to establish an association between an odour and a reward.

However, this effect was not carried through to the evaluation stage (Fig. 1C). The conditioned response towards the trained odour alone measured 15 min after acquisition did not differ between GLY concentrations (G-test: G_H=0.550, P=0.760, N=159, d.f.=2; Fig. 1C). Overall, these results show that a prolonged exposure to sub-lethal concentrations of GLY does not have an effect on the establishing of short-term memories, but it does impair the ability to establish odour–reward associations, which could be related to the detrimental effect found on gustatory responsiveness.

Effect of acute exposure to glyphosate on hive-reared bees

Elemental olfactory learning

After studying the effects of a prolonged exposure to GLY at pre-foraging ages, we wondered whether an acute exposure to GLY at foraging ages could also have an effect on honeybees. We started by performing an elemental PER conditioning assay with 0 or 2.5 mg GLY per litre of 1.8 mol l^{-1} sucrose solution as reward. Fig. 2 shows the overall performance of both groups of bees for the duration of eight acquisition trials and five extinction trials. Right away, from trial 2 of the acquisition phase, bees that received GLY in the reward showed a lower PER towards the CS (LIO). The difference between both groups remained throughout the rest of the protocol: bees that were acutely exposed to GLY responded consistently less than bees that were not exposed (Mann–Whitney U-test: U=338.50, N_1=N_2=32, Z=2.33, P=0.019; Fig. 2).

Non-elemental olfactory learning

To further investigate acute exposure effects of GLY on hive-reared bees, we carried out a non-elemental PER conditioning assay using a negative patterning discrimination assay. Fig. 3A shows %PER averaged across all trials of A+ (LIO or 2-octanol), B+ (1-hexanol or limonene) and AB− (LIO and 1-hexanol, or 2-octanol and limonene), for each group of bees exposed to a different GLY concentration. A GLY concentration × element (2×2) ANOVA yielded no differences for the elements A+ versus

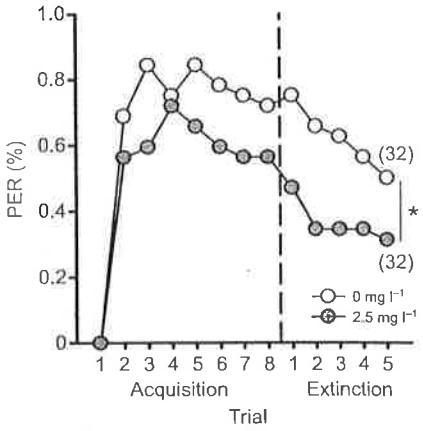

Fig. 2. Effect of acute exposure to GLY on elemental olfactory learning in honeybees. Learning abilities of bees captured at the hive entrance and exposed acutely to GLY were tested through an absolute classical conditioning procedure. The PER (%) towards the trained odour was quantified over the course of eight acquisition and five extinction trials in which the unconditioned stimulus consisted of either 1.8 mol l^{-1} sucrose solution or a compound of 1.8 mol l^{-1} sucrose solution and 2.5 mg GLY per litre of sucrose solution. The switch from acquisition to extinction occurred on trial 8. The number of bees tested is shown in brackets beside each curve (Mann–Whitney: *P<0.05).

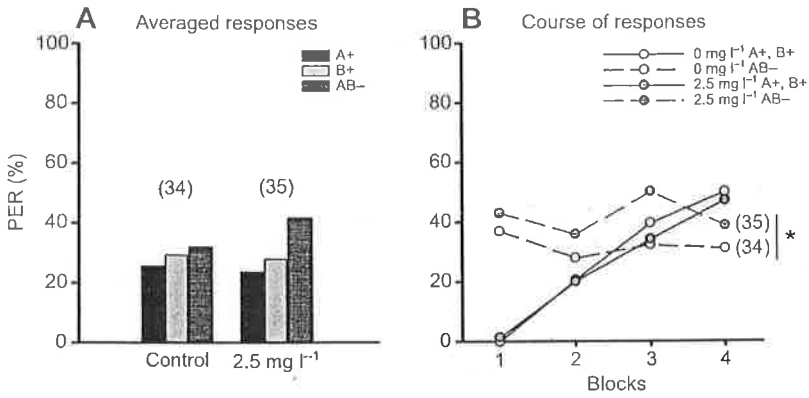

Fig. 3. Effect of acute exposure to GLY on non-elemental olfactory learning in honeybees. Non-elemental learning abilities of bees captured at the hive entrance and exposed acutely to GLY were tested through a negative patterning olfactory conditioning procedure in which the unconditioned stimulus consisted of either 1.8 mol l^{-1} sucrose solution or a compound of 1.8 mol l^{-1} sucrose solution and 2.5 mg GLY per litre of sucrose solution. (A) Averaged %PER across all trials of A+, B+ and AB− for both groups. (B) Course of %PER to the reinforced elements (A+, B+; solid line) and to the non-reinforced compound (AB−; dashed line) for both groups. Trials were grouped into four blocks of two CS+ (one A+ and one B+) and two CS− trials each. The number of bees in each group is shown in brackets above each bar (A) and beside each curve (B) [*P<0.05 (two-way ANOVA); n.s., no significant differences].

B+ (two-way ANOVA: $F_{1,134}$=0.82, P=0.367; Fig. 3A). We therefore pooled the reinforced elements (A+ and B+) within each GLY group for the next analysis. Fig. 3B shows the course of conditioned responses to the compound AB− and the average responding to the elements A+ and B+ across blocks of trials for each group. Bees in both groups could correctly discriminate the reinforced elements (A+, B+) from the non-reinforced element (AB–), as shown by the increase in response towards the reinforced elements throughout the trials whilst the response to the non-reinforced element remains constant. We then evaluated total acquisition (and therefore overall amount of differentiation) by computing the average level of responding to the pooled CSs+ and to the CS− for each GLY group. Bees rewarded with GLY during the negative patterning discrimination assay had an overall lower acquisition than non-exposed bees (two-way ANOVA: $F_{1,134}$=5.92, P=0.016; Fig. 3B).These results indicate that an acute exposure to sub-lethal GLY concentrations impairs non-elemental learning abilities of hive-reared bees.

Foraging-related behaviour

We investigated the effects of an acute GLY exposure in a more realistic and natural context by training bees to an artificial feeder and measuring different foraging variables for each bee, before and after the artificial feeder contained a sucrose solution with GLY. We started by analysing the cycle time (min) and visit frequency (cycles h^{-1}) of each bee, before and after the exposure. Bees continued visiting and collecting at the artificial feeder at a constant rate regardless of whether the artificial feeder contained GLY (Wilcoxon matched pairs test; cycle time: Z=1.15, N=6, P=0.249; Fig. 4A; visit frequency: Z=1.57, N=6, P=0.116; Fig. 4B).

Having established that foragers return to the hive and complete foraging cycles in the same manner even when GLY is present at the food source, we then focused on the transfer of information that occurs inside the hive. Dance probability did not differ before or after GLY exposure (Wilcoxon matched pairs test; dance probability: Z=0.944, N=9, P=0.345; Fig. 4C). Thus, we assayed the dance event in itself. We found no change in the mean number of waggle runs

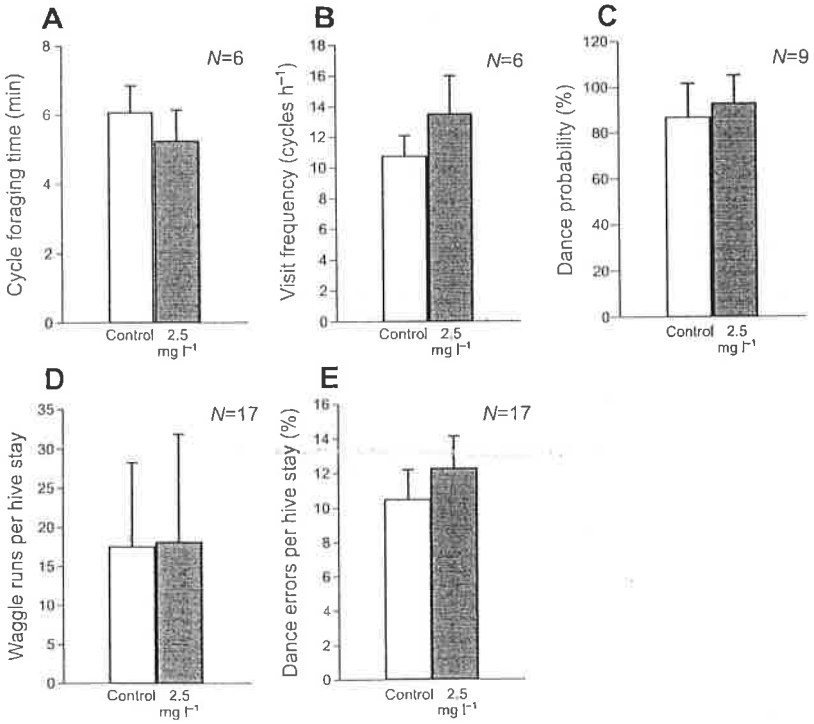

Fig. 4. Effect of acute exposure to GLY on foraging and dancing behaviour in honeybees. (A) Cycle foraging time (min); (B) visit frequency to the feeder (foraging cycles per hour); (C) dance probability (%); (D) number of waggle runs displayed per hive stay; and (E) dance errors per hive stay (%). The reward programme consisted first of three foraging bouts in which single foragers were collected at a feeder located 150 m from the hive, which offered a 2 mol l^{-1} sucrose solution without GLY (control). On the fourth visit and for the next three bouts, the sucrose solution contained 2.5 mg of GLY per litre of sucrose solution. Bars indicate means ± s.e.m. The number of bees evaluated for each variable is shown in the top right corner of each graph. There were no significant differences between the control and the treatment.

per hive when GLY was added to the food source (Wilcoxon matched pairs test: Z=0.024, N=17, P=0.981; Fig. 4D). The mean percentage of dance errors per hive stay was not affected either by the presence of GLY in the sucrose solution (Wilcoxon matched pairs test: Z=0.639, N=17, P=0.523; Fig. 4E).

DISCUSSION

We set out to evaluate the effects of chronic and acute exposures to field-realistic doses of GLY, the main herbicide currently used for weed control in agriculture, on the behaviour of the honeybee *A. mellifera*. Our results show that both chronic and acute exposure to GLY traces produce sensory sensitivity and cognitive deficits on adult honeybees of the worker caste. The concentrations used (within a 0 to 3.7 mg a.e. l^{-1} range) were based on concentrations recommended for spraying and on those measured in natural environments, from 1.4 to 7.6 mg a.e. l^{-1} (Goldsborough and Brown, 1988; Feng et al., 1990; Giesy et al., 2000), and were shown to be sub-lethal for honeybees. Young adult bees chronically exposed to concentrations of 2.5 and 5.0 mg l^{-1} of GLY showed reduced sensitivity to sucrose (reward) and impaired acquisition dynamics during elemental associative olfactory learning. This impairment cannot be explained by deterioration of the general state or motor skills of the subjects, as measurements such as survival, food uptake and locomotive activity did not differ between experimental groups. Furthermore, acute exposure to GLY significantly decreased short-term memory retention and negatively affected non-elemental associative learning at foraging ages. Nevertheless, an acute exposure to GLY in a foraging context did not have a detrimental effect on foraging activity and dancing behaviour. Altogether, these results imply that GLY at concentrations that can be found in nature as a result of standard spraying reduce sensitivity to nectar reward and also impair associative learning in honeybees. Because no effect on foraging activity was found, successful forager bees can become a source of inflow of nectar with GLY traces into the hive, which in turn could have long-term negative consequences on colony survival.

Our first results shed light on the effects of a prolonged exposure to sub-lethal concentrations of GLY during the first 15 days of adult honeybee life. An exposure to GLY during this period caused both a lower sensitivity to reward and a reduction in the dynamics of acquisition without an effect on memory retention, compared with non-exposed bees. One plausible explanation for these results is that a prolonged exposure to GLY promotes an increase in sugar response thresholds and that this is expressed by a lower PER percentage to the rewarded odour during training. There is evidence that sub-lethal concentrations of insecticides such as neonicotinoids can in fact affect behaviours involved in honeybee foraging; for example, the sugar response thresholds that increase with traces of these insecticides (Eiri and Nieh, 2012) and impair learning and memory processes (Williamson and Wright, 2013; Fischer et al., 2014). However, we have not found any record of similar effects due to the use of herbicides. It is important to note that survival and behavioural variables after a prolonged exposure to GLY show that all bees, independently of whether they had been exposed to GLY and of the GLY concentration to which they were exposed, had a similar general state at 15 days of age.

With respect to the acute exposure of adult bees to the herbicide, we also showed that honeybees present a diminished capacity to associate an odour with a reward through elemental associative learning, as was observed through exposure to a low GLY concentration (2.5 mg l^{-1}). Furthermore, acute exposure to GLY shows effects not only on the acquisition of an odour–reward association, but also on retention of olfactory memory. This can be deduced by the faster extinction process found in bees trained with reward that contained sub-lethal concentrations of GLY. Moreover, we found a similar deficit when we exposed bees to GLY during a non-elemental associative learning protocol that requires a more complex cognitive process. Even though the response towards the unrewarded mix of odours (AB–) did not decay along conditioning as was expected (Giurfa, 2003), the differences between PER values towards rewarded and unrewarded stimuli along the learning process were increasingly higher for untreated bees. Consequently, a negative patterning learning paradigm can be better resolved without the presence of the herbicide in the reward. Overall, these results suggest that an acute exposure to GLY affects the nervous system of bees either by acting on chemo-sensory stimuli perception (gustatory and/or olfactory) or by directly hindering the association between the unconditioned and the conditioned stimulus. In both cases, individuals exposed to this herbicide would need more learning events in order to reach response levels similar to those not exposed.

Honeybees roam the countryside when foraging. During their trips, they interact both with plants that are targeted by agrochemical spraying and with non-target plants that have become contaminated by drift or accidental spraying. They do not always identify foreign substances in nectar as noxious and so continue gathering it. Subtle negative effects promoted by handling nectar with GLY traces may impair important processes that play a fundamental role in the framework of foraging activities, such as response thresholds for reward and odour–reward learning. When we evaluated the behaviour of free-flying bees, focusing specifically on foraging and recruitment behaviour (measured through the waggle dance), we found no effect when we added traces of GLY to an artificial food source. In fact, honeybees neither interrupted foraging activity nor were they impeded from intensely displaying a complex motor pattern such as the waggle dance once back in the hive. This result is consistent with the lack of effect on locomotive activity after a prolonged exposure to GLY.

The constant inflow of GLY into the hive means that the agrochemical would accumulate in the hive's stores, which would then be fed to larvae and young bees and used as sustenance for the whole colony during the winter. A recent study found no effects of GLY on brood survival, development or mean pupal mass in a realistic exposure scenario (Thompson et al., 2014). In that study, honeybee colonies were exposed to the herbicide when the glasshouse where the colonies were settled was sprayed with GLY (i.e. higher glyphosate doses would go into the hive than in the present study). Despite these results, bees chronically exposed to GLY or any other agrochemical found in the food sources of the hive may perform tasks with diminished cognitive capacities, as we showed in this study. Therefore, it is likely that activities that require a decision-making process based on information previously acquired through learning and memory, such as which nectar to process (Goyret and Farina, 2005), which dances to follow (Balbuena et al., 2012a) or which source to visit (Balbuena et al., 2012b), will be affected. This in turn might have negative consequences on the search and collection of resources as well as on the coordination of collective activities. In the long term, this could affect the survival of these colonies.

Our results have shown that the presence of sub-lethal concentrations of GLY in this context has the following consequences: (1) a lower sensitivity to reward, (2) the formation of weak associative memories that can be extinguished rapidly and (3) a difficulty in establishing non-elemental associations. These difficulties in establishing associative memories would, in turn, make the gathering of resources inefficient. However, our results

have also shown that foraging behaviour is not immediately affected by the presence of GLY in the food source. Therefore, these same forager bees become vectors of the herbicide that is taken back to the hive, disseminated between the individuals of the hive and stored in their reserves for long periods of time (Kirchner et al., 1988).

Bearing in mind the results we found regarding the effects of GLY on sensory sensitivity and associative learning, it is hard not to wonder what effect GLY has on the survival and sanitary state of honeybee hives exposed to this agrochemical. This is the first study on the sub-lethal effects of an herbicide on honeybee behaviour and we hope it contributes to understanding how honeybee hives situated in agricultural environments are affected by agrochemicals. Many questions fan out from our results. For instance, how would honeybees exposed to sub-lethal doses of GLY be affected by experiencing stress from infestation with parasites or pathogens? Could an exposure to a combination of a pesticide and GLY have a synergistic effect on honeybees? What are the mechanisms underlying the effects found in the present study? It is therefore essential to examine the real exposure of honeybees to GLY in agricultural environments in order to determine to what extent chronic exposure is likely and what risks it actually implies for honeybee colony survival.

MATERIALS AND METHODS

Study site and animals

Experiments were performed during the austral spring, summer and fall seasons between 2010 and 2013. European honeybees, *A. mellifera*, of the worker caste were reared either in the laboratory or in hives from our apiary located at the experimental field of the University of Buenos Aires, Buenos Aires, Argentina (34°32′S, 58°26′W).

To study the effect of prolonged exposures to GLY, we worked with adult bees reared under laboratory conditions (laboratory-reared bees). Bees were obtained from sealed brood frames placed in an incubator [36°C, 55% relative humidity (RH) and darkness]. Recently emerged adults (0–1 days old) were collected in groups of approximately 100 individuals in wooden cages (10×10×10 cm) that had a wire mesh door on one side. Bees were fed with a 1.8 mol l^{-1} sucrose solution with different GLY (Sigma-Aldrich, Steinheim, Germany) concentrations, in addition to water and pollen *ad libitum*. Three GLY concentrations were used: 0 (control group), 2.5 and 5 mg l^{-1} of sucrose solution. Caged bees were kept in an incubator (31°C, 55% RH and darkness) until 15 days of age. Feeding tubes were refilled every 48 h in order to reduce any effects that high incubator temperatures might have on GLY and to avoid bacterial proliferation, which is known to shift the pH in sucrose solutions.

Experiments to study the effect of acute exposures to GLY were performed using worker bees caught at the entrance of outdoor hives at the beginning of each experimental procedure (hive-reared bees). In order to study foraging-related behaviour, a colony of 3000 to 4000 worker bees, queen and brood was placed in a two-frame observation hive (von Frisch, 1967) located inside the laboratory. The experimental hive consisted of two transparent acrylic walls and had a lateral opening so that bees could forage freely. Individually labelled colony bees [with plastic tags on thorax, *Opalithplättchen* (von Frisch, 1967), or with acrylic paint marks] were trained to forage on a feeder more than 100 m away from the hive. To ensure that marked individuals belonged to the experimental colony, those bees with marks that were not seen inside the observation hive were captured at the artificial feeder and removed from the experiment.

The experiments comply with the 'Principles of animal care', publication No. 86-23, revised 1985 of the National Institutes of Health, and also with the current laws of the country in which the experiments were performed.

Experimental series

Effect of prolonged exposure to GLY on laboratory-reared bees

To study the effect of a prolonged exposure to GLY, we evaluated post-exposure locomotive activity, sensitivity to sucrose and olfactory PER conditioning as well as survival and food ingestion during the 2 week experimental period.

Survival, food ingestion and locomotive activity

Mortality and food intake were quantified for all the laboratory-reared groups exposed to different GLY concentrations during the complete laboratory rearing period (15 days). These recordings were carried out to corroborate whether GLY concentrations were sub-lethal. In order to quantify mortality, the number of dead bees per cage was recorded daily (and dead bees were removed). To quantify food intake, the volume of solution remaining in the feeding tubes was recorded daily for each cage and calculated relative to the number of bees alive each day. Additionally, other variables were measured to evaluate the general state of sensory sensitivity and locomotive activity in bees after a prolonged exposure to GLY. First, spontaneous response to an unconditioned stimulus was measured as follows: the antennae of test bees were touched with a drop of 1.8 mol l^{-1} sucrose solution and the number of responses was recorded. Mortality between harnessing and the conditioning protocol was also measured.

We used an adapted protocol to record the locomotive and orientation activity of 17-day-old bees (Rueppell et al., 2007). Each bee was taken from the cage and introduced into a darkened circular arena that had a video camera (Sony Handycam HDR-SR11) on infrared mode located on the top section and four LED lights at equal distances around the perimeter. Four lights of two different colours were placed equidistantly around the arena, alternating colours so lights of the same colour pair faced each other. After an initial acclimatization of 2 min, the first light was turned on until the bee oriented and moved towards it. Once the bee was in the vicinity of the first light, the light was turned off and the one opposing it was turned on. This was repeated sequentially (first a green light, then the opposing green light, then a yellow light and finally the opposing yellow light) until the bee had visited all lights twice. The time taken by each bee to complete the circuit was recorded using a self-written event-recording program, and then discriminated by LED colour.

Sensitivity to sucrose

Individuals exposed to GLY during the first 15 days of the adult stage were taken from their cages, anaesthetized at 4°C and harnessed on plastic holders that restrained body movement but allowed free movement of antennae and mouthparts (Page et al., 1998). After awakening, bees were offered water to drink and housed in an incubator (30°C, 55% RH and darkness) for at least 1 h before the protocol was carried out. In order to measure sensitivity to reward, the antennae of test bees were stimulated with droplets of sucrose solution of increasing concentration. Prior to performing a PER–GRS assay (Page et al., 1998; Scheiner et al., 1999), water was offered again in order to avoid confounding thirst effects. PER was quantified as bees were presented with sucrose solutions of increasing concentration (0.1, 0.3, 1, 3, 10, 30 and 50% w/w). The lowest sucrose concentration at which an individual responded by extending its proboscis was interpreted as its sugar response threshold. Bees were lined up in groups of 20–35 individuals and tested for each concentration sequentially, i.e. all bees were tested first at 0.1%, then at 0.3%, and so on. All bees were tested for their response to water between each concentration of sucrose solution. This serves to control for potential effects of repeated sucrose stimulation that could lead to increased sensitization or habituation. The inter-stimulus interval between water and sucrose solution depended on the number of individuals tested at a given time, but averaged 3 min. At the end of the procedure, a GRS was obtained for each bee. This score was based on the number of sucrose concentrations to which the bees responded (which correlates with the sugar response threshold because bees normally respond to all concentrations above their threshold). The response was arbitrarily quantified with scores from one to seven, where one represented a bee that only responded to one concentration of sucrose (usually 50% w/w), while a score of seven represented an individual that responded to all concentrations tested. If a bee failed to respond to sucrose concentration in the middle of a response series (e.g. responded to 0.1, 0.3, 3 and 10% w/w, but did not respond to 1%), this 'failed' response was considered to be an error and the bee was deemed to have responded to that concentration as well. A bee that did not respond to any of the sucrose concentrations (score of 0) was excluded from further

analyses. In addition, those bees that responded to all sucrose concentrations and all presentations of water were excluded from analyses as they appeared not to be able to discriminate between sucrose solution and water.

Olfactory PER conditioning

After an exposure to GLY during the first 15 days of the adult stage, individuals were taken from their cages, anaesthetized and harnessed as described above and kept in an incubator (30°C, 55% RH and darkness) for approximately 2 to 3 h before the protocol of olfactory PER conditioning (Takeda, 1961; Matsumoto et al., 2012) was carried out. During classical conditioning, a constant airflow of 50 ml s^{-1} was delivered to the head of bees through a tube (1 cm diameter) placed 2 cm in front of the bee, using an electronic device. A 30×9×3 mm piece of filter paper was impregnated with the odour (4 µl of a pure odorant, LIO) and placed inside a syringe located in the electronic device to add the odour to the airflow when required. The volatile was delivered through a secondary air stream (6.25 ml s^{-1}) injected in the main airflow during the delivery of the odour. During the experiment in the PER setup, a fan extracted the released odours to avoid contamination. Before odour presentation, bees were left to rest for 15 s in the airflow for familiarization as well as for testing their response towards the mechanical stimulus. Only bees that showed the unconditioned response after applying 50% w/w (1.8 mol l^{-1}) sucrose solution onto the antennae and that did not respond to the mechanical stimulus (airflow) were used. For the training procedure, the PER towards the trained odour (%PER) was quantified over the course of three acquisition trials. We presented the CS (LIO) for 6 s and each learning trial lasted 40 s. Reinforcement (1.8 mol l^{-1} sucrose solution without GLY) was presented on the proboscis and occurred for 3 s, 3 s after the onset of the CS. The conditioned response towards the trained odour on its own (test) was measured 15 min after acquisition by quantifying PER during the first 3 s of a single presentation of the test odour (LIO).

Effect of acute exposure to GLY on hive-reared bees

To study the effect of acute exposure to GLY, we evaluated learning abilities in worker bees caught at the entrance of outdoor hives. The foraging-related behaviours were tested in free-flying bees that were collected at an artificial feeder.

Elemental olfactory learning

Individuals were anaesthetized and harnessed as described previously. For this experimental procedure PER towards the trained odour was quantified over the course of eight acquisition trials (%PER). Reinforcements consisted of 0 mg l^{-1} GLY or 2.5 mg $^{-1}$ GLY per litre of 1.8 mol l^{-1} sucrose solution and were presented on the proboscis. Extinction of the conditioned response was evaluated by quantifying PER to LIO over the course of five trials in which the CS was presented without any reward. Extinction followed 15 min after acquisition. Experimental setup, CS, reward times and criteria for discarding individuals were defined as described previously.

Non-elemental olfactory learning

This experimental procedure was based on a negative patterning (A+, B+, AB−) non-elemental conditioning protocol (Deisig et al., 2001). In this procedure, elements A and B were rewarded with either 0 or 2.5 mg GLY per litre of 1.8 mol l^{-1} sucrose solution (reinforced elements A+ and B+) whilst the compound AB was not rewarded (non-reinforced element AB−). This assay incorporates an additional complexity for the bee because the discrimination between elements cannot be achieved through an elemental solution, it can only be solved by recognising a certain rule. Individuals were anaesthetized and harnessed as described previously. The CSs were the odorants LIO and 1-hexanol for one group of bees and limonene and 2-octanol for another (Sigma-Aldrich, Steinheim, Germany). We only report analyses of the pooled data. The experimental setup and reward times were as described previously. In this case, during periods of odorant delivery, the airflow was shunted through a syringe containing the odorant. In that way, a single odorant or a compound of two odorants could be delivered to the bee. In the latter case, the valves corresponding to two different syringes were opened simultaneously so the airflow arriving at the antennae of the bee contained the two odours as a compound. PER was quantified over the course of the protocol, both for reinforced and non-reinforced trials. Non-reinforced trials consisted of 6 s CS presentation without reward. After experiments were finished, all animals were again tested for PER. If an animal did not respond, it was discarded from further analyses (<10%). All bees received a total of 16 training trials: four A+ trials, four B+ trials and eight AB− trials. The sequence of CS+ and CS− trials was randomized.

Foraging-related behaviour

The experiment consisted of six successive visits to the artificial feeder for each bee. During the first three visits, the feeder offered 2 mol l^{-1} sucrose solution without GLY. During the last three visits, the solution was changed to 2.5 mg l^{-1} GLY per litre of 2 mol l^{-1} sucrose solution. At the observation hive, we video recorded (Sony Handycam HDR-SR11) the behaviour of the returning foragers during all visits. Data were obtained from video and quantified using a self-written event-recording program. Five variables were evaluated for each bee: (1) cycle time (min) taken by a forager to arrive to the feeder, collect, fly back to the hive and leave the hive for the next cycle, calculated as the time between the first and final visits, over the total number of cycles completed; (2) visit frequency (feeding cycles h^{-1}), calculated as the inverse of the cycle time; (3) dance probability (%), calculated as the number of hive visits in which a dancing event was recorded, over the total number of complete hive visits; (4) mean number of waggle runs per hive stay, calculated as the number of waggle phases completed for each complete hive stay, over the total number of complete hive visits; and (5) dance errors per hive stay (%), calculated as the number of correct and incorrect turns for all the dances of each bee, over the total number of complete hive visits. For this latter measurement, when a forager performs a waggle dance, she normally turns alternately to the left or the right to begin the return phase at the end of the waggle phase (von Frisch, 1967). Deviations from the alternate left and right turns (e.g. two consecutive right turns) appear to be a measure of how disordered the dance is.

Statistical analysis

Mortality is expressed as percentage accumulated mortality for the complete exposure period per cage. Cumulative food intake is expressed as cumulative millilitres per bee. The means of mortality (percentage accumulated mortality for the complete exposure period per cage) and of food intake (cumulative millilitres of food ingested per bee) were analysed using a one-way ANOVA (Sokal and Rohlf, 1995). Normality and homoscedasticity assumptions were met for all data. Mortality between harnessing and the conditioning protocol for the different GLY concentrations was analysed through a *G*-test of homogeneity. Time taken by bees exposed to different GLY concentrations between each pair of LED lights in the locomotive and orientation procedure was analysed using a three-way repeated-measures ANOVA (RM-ANOVA) with GLY concentration and LED colour as fixed factors and cage and bees as random factors. Data met normality, homogeneity and sphericity assumptions after $\log_{10}$ transformation.

GRS data were treated as nonparametric because the assumption of normality was not met. Median GRSs were compared between GLY concentrations using Kruskal–Wallis ANOVA tests.

PER proportions for each GLY concentration during each acquisition trial were assayed using RM-ANOVA. Monte Carlo studies have shown that it is possible to use ANOVA on dichotomous data (Lunney, 1970). Where necessary, simple effects were computed and Tukey tests were used to perform *post hoc* comparisons. PER proportions for each GLY concentration towards the trained odour on its own (test) were assayed using a *G*-test of homogeneity.

PER for the different GLY concentrations throughout acquisition and extinction (elemental learning procedure) were analysed by assigning a value to each bee corresponding to the total number of trials during which they exhibited PER across the 13 trials of the procedure. This value, which ranged from zero to 13, was assayed using a Mann–Whitney *U*-test for independent samples to compare overall performance levels between groups (Zar, 1999).

The percentage of conditioned responses (%PER) in successive CS+ trials (omitting the randomly interspersed CS− trials) and in successive CS− trials (omitting the randomly interspersed CS+ trials) were measured for the non-elemental learning procedure. Bees were subjected to four A+, four B+ and

eight AB− trials. Data were grouped to obtain four blocks of two CS+ trials and four blocks of two CS− trials. A two-way ANOVA was used for comparisons between elements and a further two-way ANOVA was used for comparisons between GLY concentrations. Monte Carlo studies have shown that it is possible to use ANOVA on dichotomous data (Lunney, 1970).

Finally, all foraging variables were analysed in the same manner. A mean for the first three visits and a mean for the last three visits were obtained for each bee. Means for each variable were compared using a Wilcoxon matched pairs test (Zar, 1999).

The alpha level was set to 0.05 for all analyses.

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Competing interests

The authors declare no competing financial interests.

Author contributions

L.T.H., A.A. and W.M.F. conceived and designed the experiments. L.T.H., D.E.V. and A.A. performed the experiments. L.T.H. and D.E.V. performed data analysis. L.T.H., A.A. and W.M.F. drafted the manuscript. All authors revised and commented on the manuscript.

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RESEARCH ARTICLE

Effects of sublethal doses of glyphosate on honeybee navigation

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ABSTRACT

Glyphosate (GLY) is a herbicide that is widely used in agriculture for weed control. Although reports about the impact of GLY in snails, crustaceans and amphibians exist, few studies have investigated its sublethal effects in non-target organisms such as the honeybee *Apis mellifera*, the main pollen vector in commercial crops. Here, we tested whether exposure to three sublethal concentrations of GLY (2.5, 5 and 10 mg l^{-1}; corresponding to 0.125, 0.250 and 0.500 µg per animal) affects the homeward flight path of honeybees in an open field. We performed an experiment in which forager honeybees were trained to an artificial feeder, and then captured, fed with sugar solution containing traces of GLY and released from a novel site either once or twice. Their homeward trajectories were tracked using harmonic radar technology. We found that honeybees that had been fed with solution containing 10 mg l^{-1} GLY spent more time performing homeward flights than control bees or bees treated with lower concentrations. They also performed more indirect homing flights. Moreover, the proportion of direct homeward flights performed after a second release from the same site increased in control bees but not in treated bees. These results suggest that, in honeybees, exposure to levels of GLY commonly found in agricultural settings impairs the cognitive capacities needed to retrieve and integrate spatial information for a successful return to the hive. Therefore, honeybee navigation is affected by ingesting traces of the most widely used herbicide worldwide, with potential long-term negative consequences for colony foraging success.

KEY WORDS: *Apis mellifera*, Glyphosate, Sublethal effects, Navigation, Harmonic radar tracking

INTRODUCTION

Honeybees (*Apis mellifera*) are the main pollinators in agricultural settings (Aizen et al., 2009) and as such are highly exposed to any perturbation occurring in the surroundings of crop fields. Consequently, this eusocial insect can serve as a biosensor to accurately determine environmental pollutants (Devillers and Pham-Delègue, 2002). Any foreign substance present in gathered resources (i.e. pollen and nectar) may also be stored and accumulated inside the nest for long periods, potentially affecting nest mates of all stages (Devillers and Pham-Delègue, 2002). This applies in particular to highly water-soluble agrochemicals such as the herbicide glyphosate *N*-(phosphonomethyl) glycine, which may remain on crops after application for long periods (Zhang et al., 2011). Any subsequent accumulation of agrochemicals inside the hive could have negative effects which are often inconspicuous in the short term (Giesy et al., 2000), but which could impair individual behaviors and social organization in the long term (Kirchner, 1999).

The use of glyphosate (GLY) as a broad-spectrum post-emergent herbicide for weed control has spread rapidly in the last few decades (Goldsborough and Brown, 1988) to become one of the most commonly used agrochemicals worldwide (Zhang et al., 2011). The typical methods of administration involve spraying it directly onto foliage and aerial application (Giesy et al., 2000). As a consequence, traces of the herbicide can also be found in the surroundings of fields cultivated with the target crop. GLY deters plant growth by inhibiting an aromatic amino acid pathway that is apparently present only in plants, microorganisms and fungi, not animals (Amrhein et al., 1980; Carlisle and Trevors, 1988; Duke et al., 1989; Franz et al., 1997).

Several studies have reported negative effects of this herbicide on vertebrates and invertebrates. GLY doses between 0.1 and 10 mg acid equivalent l^{-1} have been found to reduce growth in the earthworm *Aporrectoden caliginom* (Springett and Gray, 1992) and affect reproduction and development in the freshwater snail *Pseudosuccinea columella* (Tate et al., 1997). A negative effect has also been reported in amphibians after chronic exposure to different concentrations of glyphosate (3.8–18 mg l^{-1}; Howe et al., 2004; Relyea, 2005a,b). Despite these findings and others that report negative and lethal effects on invertebrates such as amphipods (Dutra et al., 2011), the sublethal impacts of GLY on non-target organisms such as insect pollinators have so far been poorly evaluated (Herbert et al., 2014; Thompson et al., 2014). In this study, we used sublethal concentrations of GLY ranging from 2.5 to 10 mg l^{-1}.

Honeybees show a behavioral repertoire that allows the evaluation of perturbations in well-known stereotypical responses. The behavior in which bees protrude their probosces after being stimulated by applying sucrose solution to their antennae is one of these responses, and it can be used to test the effects of environmental pollutants on appetitive behavior (Devillers and Pham-Delègue, 2002). A recent study found that a concentration of glyphosate (2.5 mg l^{-1}), within the recommended range for aquatic and terrestrial weed control (Giesy et al., 2000), affects gustatory responsiveness and learning performance in harnessed bees [tested with proboscis extension response (PER) assays]. However, no effect was observed on locomotive activity when foragers collected sucrose solution contaminated with the herbicide at an artificial feeder, suggesting that GLY may accumulate inside the hive (Herbert et al., 2014). Also, Herbert and co-workers (2014) found that an acute exposure to sublethal GLY concentrations offered during olfactory PER conditioning decreased short-term memory and impaired more complex forms of associative learning in foragers.

Studies have already shown that other agrochemical compounds used for pest control, such as neonicotinoids, negatively affect honeybee gustatory sensitivity and even their dance maneuvers (Eiri and Nieh, 2012). Non-lethal doses of imidacloprid (75–

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The Journal of Experimental Biology

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1000 ppb), a neonicotinoid insecticide, which acts on cholinergic pathways of insect synaptic transmission (Gauthier, 2010), affect homing abilities (Bortolotti et al., 2003) and impair the retrieval of memory acquired during exploratory orientation flights (Fischer et al., 2014).

Honeybees are well established as a model for studies on animal navigation (von Frisch, 1967; Menzel et al., 2012; Menzel, 2012). In a typical catch-and-release experiment, bees are displaced within a previously explored area to evaluate their homing behavior using different tracking technologies (Decourtye et al., 2011; Schneider et al., 2012; Fischer et al., 2014). Exploration during orientation flights familiarizes bees with the sun compass, their distance measure (odometer) and the landmarks in the environment (Menzel et al., 2005, 2012). Further information about the landscape is added during flights between the hive and the feeding sites. Integration of the multiple sources of spatial information leads to a reference memory that allows bees to perform shortcuts between important locations (hive, feeding sites and release sites). As a result, honeybees are able to refer to a common frame of spatial reference that allows them to return to the hive even from an unfamiliar location by taking novel shortcuts (Menzel et al., 1998).

Although the GLY concentrations recommended for weed control as well as those previously detected in aquatic and agricultural systems are within the range of 1.4 mg l^{-1} to 3.7 mg l^{-1} (Couture et al., 1995; Giesy et al., 2000; Solomon and Thompson, 2003), intensive use of the herbicide during the last two decades has led to an exponential increase in the doses of GLY present in genetically modified (GM) crops (USDA data source, NASS). This situation implies that GLY concentrations found in close proximity to GM crops today should be much higher than the range previously reported. In the present study we propose that honeybees foraging on 'nectar' containing traces of GLY may have difficulty integrating complex information from their environment which they need for navigation. To evaluate whether sublethal doses of glyphosate affect *Apis mellifera* orientation and navigation, we performed a catch-and-release experiment in which honeybees flying to the hive were displaced during foraging trips.

RESULTS

In a catch-and-release experiment as performed here, we expect that bees captured at the feeder and then released from the release site (RS) are motivated to return to the hive. After ingesting food contaminated with glyphosate, we expected that these treated bees would perform irregular homeward flights or at least take more time than untreated control bees to return to the hive. Our results show that animals either start immediately with a straight flight from the release site (Fig. 1A,B) or they perform less regular flights (Fig. 1C). Some of the straight flights follow the vector the bees would have taken if they had not been relocated to the release site. These flights were either directed towards the hive and finished at the hive, or they were directed towards the feeder and then followed the trained route from the feeder to the hive (Fig. 1A). Some of these initially straight flights at the beginning of their homing behavior were followed by a single loop before the bees return to the hive (Fig. 1B). Therefore, we distinguish between two major flight categories: direct flights (straight flight with or without one loop, Fig. 1A,B) and indirect flights (flights with loops Fig. 1C).

First release

Fig. 2 shows the proportion of bees performing different homeward flights after being relocated from the feeder to the RS and released

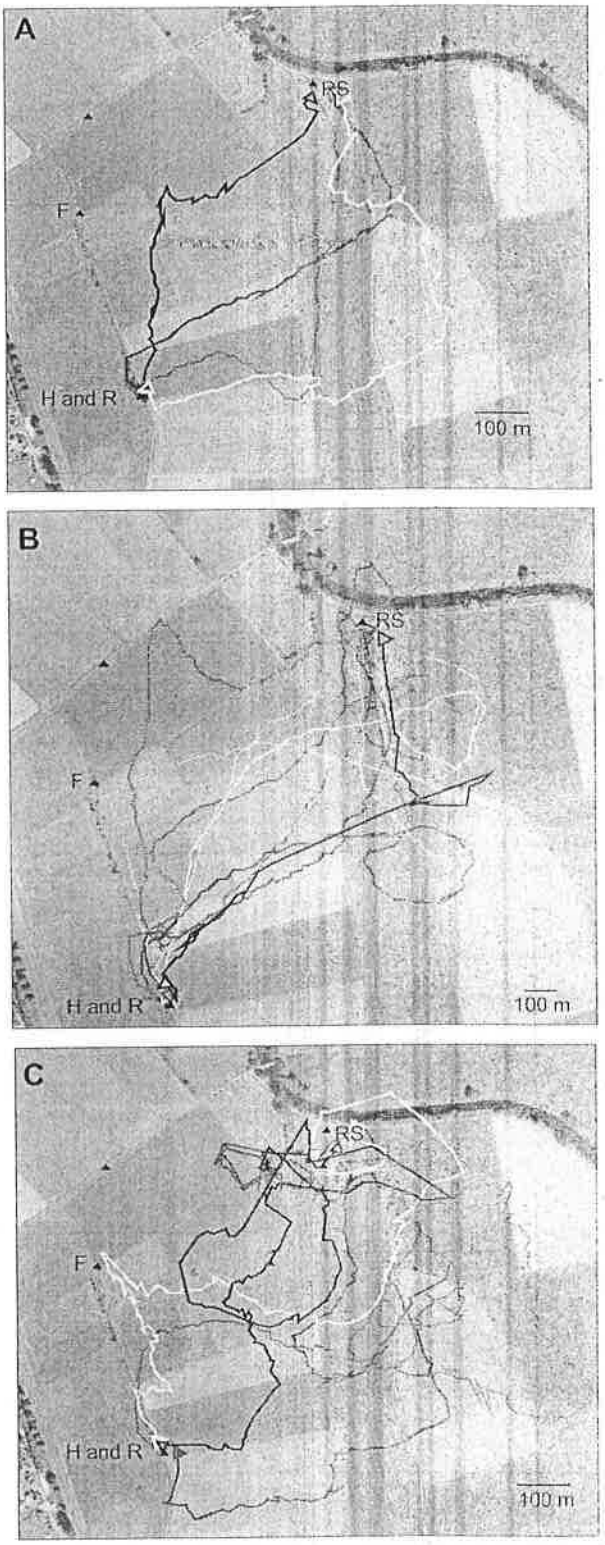

Fig. 1. Examples of homeward flights made by honeybees during the first release after treatment. Flight paths were categorized as direct (A), single-loop (B) or indirect (C). Colors: light blue and red for control bees, blue and orange for bees treated with 2.5 mg l^{-1} glyphosate (GLY), yellow and lilac for bees treated with 5 mg l^{-1} GLY, and green and gray for bees treated with 10 mg l^{-1} GLY. H, hive; R, radar; F, feeder; RS, release site.

RESEARCH ARTICLE The Journal of Experimental Biology (2015) 218, 2799-2805 doi:10.1242/jeb.117291

from the RS for the first time. As already mentioned, these homeward paths involve: (1) straight and rapid flights directly to the hive, with or without a single loop before returning to the hive ('direct flights'); or (2) irregular flights, in which bees changed direction frequently ('indirect flights'). Both control and treated bees showed similar proportions of direct flights to the hive (test of heterogeneity: χ^2=2.604; P=0.457; N=79). However, we found statistical differences in the time spent performing direct flights between treatments (Fig. 3A; Kruskal–Wallis test: H=10.008, P=0.019, d.f.=3, N=50). Specifically, bees that had ingested sucrose solution containing 10 mg l^{-1} GLY spent more time flying from the RS to the hive than control bees or bees that had ingested 2.5 or 5 mg l^{-1} GLY (Mann–Whitney test: 0 mg l^{-1} versus 10 mg l^{-1}: U=28.5, P=0.004; 2.5 mg l^{-1} versus 10 mg l^{-1}: U=13.5, P=0.016; 5 mg l^{-1} versus 10 mg l^{-1}: U=8.0, P=0.003). No statistical difference in the flight time was found between control and treated bees performing indirect flights (Fig. 3B; Kruskal–Wallis test: H=5.197, P=0.158, d.f.=3, N=29).

We observed that during some homeward flights a small number of bees passed through the feeder area. The proportion of bees that flew via the feeder was higher among control bees and bees that ingested sucrose solution with 2.5 mg l^{-1} GLY than among bees treated with 5 or 10 mg l^{-1} (see Table 1). After flying close to the feeder, those bees followed the trained flight route to the hive.

Second release

Bees learn to improve their homing flights during sequential releases from the same site (Menzel et al., 2005). Therefore, we next asked whether this form of learning is compromised in bees that have been exposed to the herbicide. To test this, bees were captured at the feeder, relocated to the RS, and released for a second time: these bees were therefore exposed twice to the same amount of GLY.

Control bees and bees that were exposed to 2.5 or 5 mg l^{-1} GLY showed a tendency to perform direct flights more frequently than indirect flights (Fig. 4). Conversely, bees that had ingested sucrose solution with 10 mg l^{-1} GLY showed the inverse tendency, with more bees performing indirect flights. Nevertheless, no statistical differences for the time spent in direct flights were found between control and treated bees (Fig. 5A; Kruskal–Wallis test: H=3.332, P=0.343, d.f.=3, N=27). It was not possible to perform a statistical analysis of data for indirect flights (Fig. 5B) because the sample size was too small (0 mg l^{-1}: N=4; 2.5 mg l^{-1}: N=1; 5 mg l^{-1}: N=2; 10 mg l^{-1}: N=3). When we compared the proportion of control and treated bees that performed direct and indirect flights after the first and second release, we found statistical differences between control bees released once or twice, but not between treated bees (Fig. 6A; Fisher's exact test: control bees, χ^2=10.80; P=0.001; treated bees, χ^2=1.07; P=0.245, N=32). Control bees modified their tendency to perform more indirect flights after the first release than after the second one, whereas the proportion of treated bees performing direct or indirect flights after one or two releases was similar. Furthermore, when studying the transitions (or lack thereof) from direct or indirect flights (or vice versa) performed after the first release to direct or indirect flights performed after the second release (direct–direct: D–D, direct–indirect: D–I, indirect–direct: I–D and indirect–indirect: I–I), we observed a tendency for control bees to perform more I–D transitions than treated bees. Interestingly, bees that had ingested the higher GLY concentration showed a tendency to perform more transitions to indirect flights (D–I, I–I) after the second release (Fig. 6B).

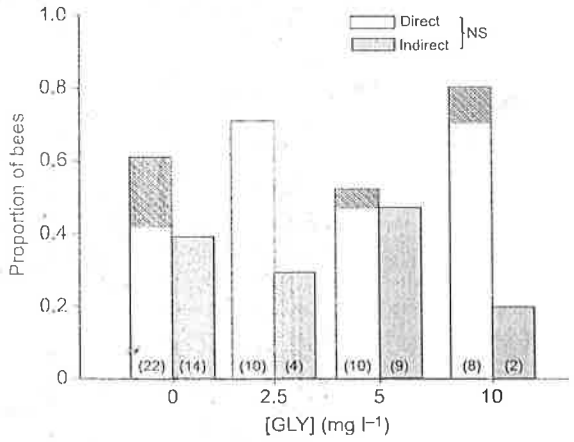

Fig. 2. Proportion of bees performing direct and indirect homeward flights after the first release. Proportion of bees performing direct and indirect homeward flights were pooled according to the treatment; looped flights are indicated by hatched bars. 0 mg l^{-1}: control bees; 2.5 mg l^{-1}, 5 mg l^{-1} and 10 mg l^{-1}: bees exposed to different concentrations of GLY (corresponding to 0.125, 0.25 and 0.5 µg per animal). NS, no significant difference (P>0.05). Numbers inside bars indicate the number of bees assessed for each treatment.

DISCUSSION

We evaluated the effect of recommended concentrations of glyphosate (GLY) used in agricultural settings on honeybee navigation (up to 3.7 mg l^{-1} GLY; Giesy et al., 2000) and two additional concentrations that are reported to be sublethal (5 and 10 mg l^{-1}). Our results show that a single exposure to a concentration of GLY within this range delays the return of the foraging honeybee to the hive. In some cases, the flight trajectories were also affected after successive exposure to the herbicide, suggesting that the spatial learning process is impaired by ingestion of the herbicide during feeding. This impairment of navigation in the explored area increased when the concentration of GLY ingested was higher. Indeed, bees fed with 10 mg l^{-1} GLY took more time to perform direct homeward flights and performed more indirect flights after the second release than bees treated with lower GLY concentrations. Bees that had ingested low concentrations of GLY (2.5 or 5 mg l^{-1}) and showed indirect flight trajectories after the first release performed direct flights after the second release. Accordingly, more experimental honeybees found the hive regardless of the herbicide concentration ingested. However, subtle effects on the homing behavior within this concentration range were seen, indicating that the GLY concentrations used in this study caused only sublethal effects on honeybees.

Regarding the kind of flight trajectories performed, we found that honeybees treated with GLY exhibited more indirect homing flights after the second release than the control bees. As reported by Menzel et al. (2005), we expected that the bees released more than once from the same location improve their homeward flights. This means that we expected a lower proportion of bees to execute indirect flights from RS to H after the second release. Our results show that a higher proportion of control bees did indeed perform indirect flights during the first release and changed to direct flights during the second one, whereas animals treated with the highest dose of GLY were impaired in terms of improving their navigation performance. Bees released twice from the RS have fed on the contaminated food twice, a fact that might promote physiological stress and/or learning impairment. We propose that both a single exposure and repeated exposures to GLY have an effect on the retrieval and formation of

The Journal of Experimental Biology (2015) 218, 2799-2805 doi:10.1242/jeb.117291

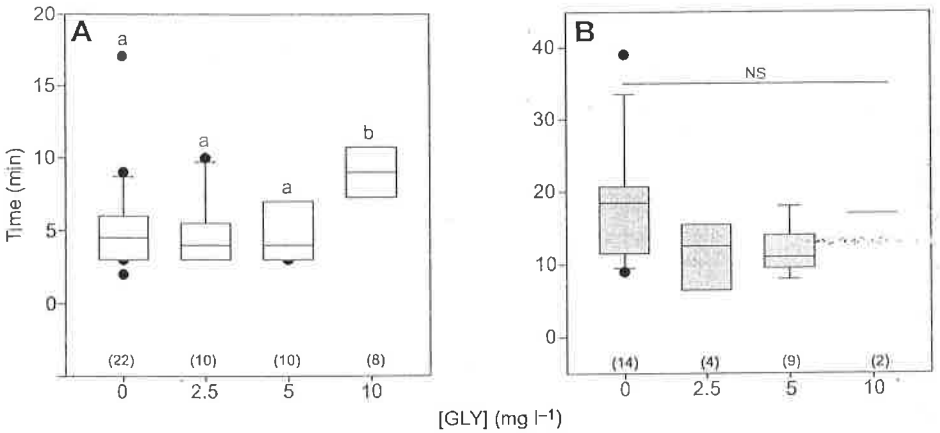

Fig. 3. Timing of homeward flights after the first release. Flying times from the release site to the hive according to different treatments (0 mg l^{-1}: control bees; 2.5 mg l^{-1}, 5 mg l^{-1} and 10 mg l^{-1}: bees exposed to 2.5 mg l^{-1}, 5 mg l^{-1} and 10 mg l^{-1} GLY, respectively). (A) Direct and 'single-loop' flights. (B) Indirect flights. Boxes with different letters are significantly different at $P<0.05$. NS, no significant differences ($P>0.05$). Numbers in brackets indicate the number of bees assessed for each treatment.

memory. The effect of GLY on memory retrieval is indicated by the reduced probability of bees taking a shortcut to the hive or the feeder, longer search flights and a lack of improvement of homing behavior after experience.

A recent study using the PER paradigm showed that acute exposure to GLY (2.5 mg l^{-1}) affect the retention of olfactory memory in honeybees evaluated in both simple and complex associative learning tasks. The learning process for both kinds of paradigm is faster for untreated bees and, specifically for a kind of negative pattern learning, in the presence of GLY in the reward (Herbert et al., 2014). Navigation requires several rather complex cognitive capacities during memory formation and retrieval that allow them to integrate current and previously acquired environmental information. These processes would be compromised by the uptake of higher concentrations of GLY used, as we show for 5 (Fig. 6) and 10 mg l^{-1} (Figs 3, 4 and 6) concentrations. A plausible explanation for this response is that the herbicide impairs appetitive behaviors, disturbing not only those processes involved in acquiring and associating chemosensory information, as proposed in a previous study (Herbert et al., 2014), but also the use of stored information about the environment acquired during the exploratory orientation flights of foragers and the experience gained from homing flights over the course of the experiment. Thus, feeding on nectar containing traces of GLY might affect the learning and retrieval of memory relevant for the recognition of food sources and for navigating between those food sources and the hive.

The ingestion of specific insecticides in sublethal concentrations increases sugar response thresholds (Eiri and Nieh, 2012) and affects homing in honeybees (Henry et al., 2012; Fischer et al., 2014). Herbert and co-workers (2014) have shown that chronic exposure to traces of GLY reduces responsiveness to sucrose and learning performance during olfactory PER conditioning. Furthermore, when honeybees were exposed to high levels of this herbicide they showed impaired associative learning, but no clear effects on their dancing behavior were observed (Herbert et al., 2014). These data support the view that exposure to GLY, even at low concentrations, negatively affects gustatory responsiveness and thus also the motivation to forage for food in free-flying honeybees in the experiments reported here. This motivational effect, however, was not strong enough to eliminate homing behavior but appeared to reduce the acquisition of new navigational memory.

The experiment performed here focused on the action of GLY over a short period of time (hours) but chronic exposure to the herbicide could have additional effects and may affect the general performance of the entire colony. Usually, genetically modified herbicide-tolerant crop fields are surrounded by native flora (Bohan et al., 2005). As we mentioned above, honeybees are the main pollinator in agricultural ecosystems, but they also play a key role in pollination of native flora (Aizen et al., 2009). As a consequence of GLY application in those agricultural crops and its drift (Chang et al., 2011) to neighboring areas, the native species in the surrounding areas could be affected (Matthews, 2006), as well as their pollinators. Moreover, in countries that have introduced glyphosate-resistant GM crops, traces of GLY were detected in honey (Chile: CIAP, 2012; Rubio et al., 2014), air particles and rain samples (USA: Chang et al., 2011; Argentina: Alonso et al., 2014) and in the surface of bodies of water located close to treated fields that could be visited by honeybees (Canada: CCME, 1989). In addition, we focus on agricultural settings and their surroundings – a

Table 1. Data for control and GLY-treated bees released for the first time

GLY treatment	No. of bees released	No. arrived at hive	No. arrived at hive via feeder	No. not arrived
0 mg l^{-1}	46	36 (0.78)	6 (0.17)	10 (0.22)
2.5 mg l^{-1}	25	14 (0.56)	6 (0.42)	11 (0.44)
5 mg l^{-1}	22	19 (0.86)	2 (0.11)	3 (0.14)
10 mg l^{-1}	15	10 (0.67)	2 (0.2)	5 (0.33)

Numbers in parentheses indicate the proportion of bees for each treatment.

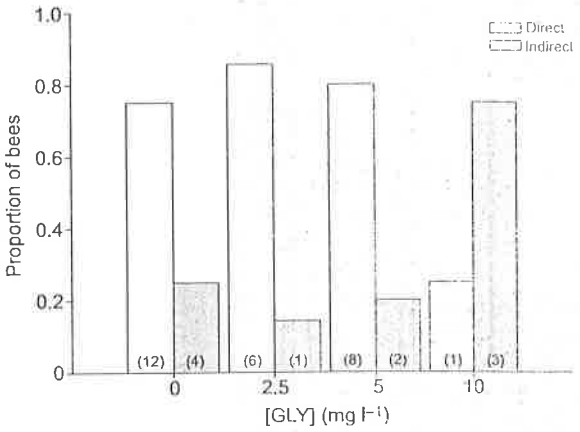

Fig. 4. Proportion of bees performing direct and indirect homeward flights after the second release. Numbers in brackets indicate the number of bees assessed for each treatment.

The Journal of Experimental Biology (2015) 218, 2799-2805 doi:10.1242/jeb.117291

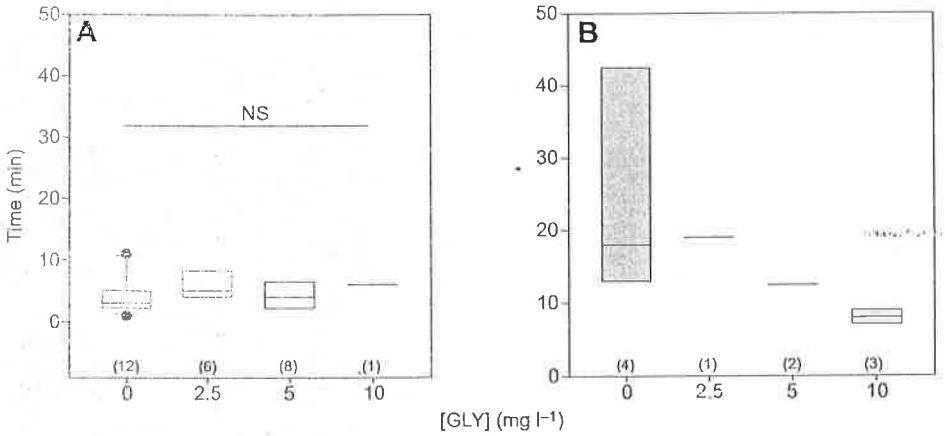

Fig. 5. Timing of homeward flights of bees after the second release. Flying times from the release site to the hive represented for the different treatments (0 mg l^{-1}: control bees; 2.5 mg l^{-1}, 5 mg l^{-1} and 10 mg l^{-1}: bees exposed to 2.5 mg l^{-1}, 5 mg l^{-1} and 10 mg l^{-1} GLY, respectively). (A) Direct flights. (B) Indirect flights. NS, no significant differences (P>0.05). Numbers in brackets indicate the number of bees assessed for each treatment.

system that includes the wild flora. The presence of GM crops in some countries where monoculture is common is often linked to the use of aerial spraying to inoculate pesticides, a situation that promotes drift of agrochemicals to non-target areas (Matthews, 2006; Chang et al., 2011). Moreover, herbicides are used beyond the surroundings of commercial crops; nowadays, its scope has reached domestic use in homes and gardens (Matthews, 2006), where honeybees can potentially collect food resources.

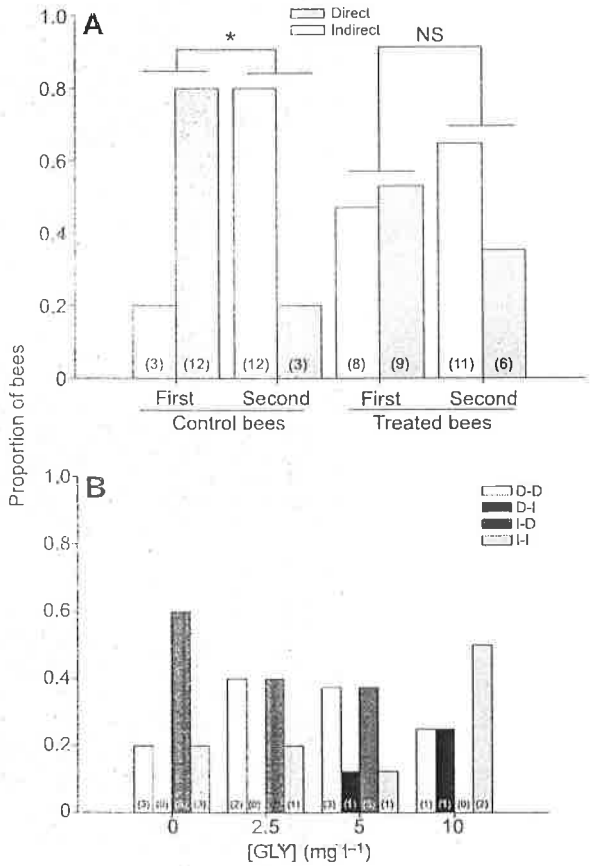

Fig. 6. Proportion of transitions in performance after the first and second release according to treatment. (A) Control and treated bees were categorized according to direct (white bars) or indirect flights (gray bars) after the first release (fed GLY once) or the second release (fed GLY twice). (B) Flight transitions between the first and second release was considered per experimental bee (D: direct flight, I: indirect flight). The categories of both flights were: D–D (both flights were direct), I–I (both flights were indirect), D–I (the first flight was direct and the second indirect), I–D (the first flight was indirect and the second direct). *P<0.05; NS, no significant difference (P>0.05). Numbers in brackets indicate the number of bees assessed for each treatment.

As the resistance of organisms to agrochemicals increases, higher concentrations are used to treat agricultural crops (ARMS, 2014), and pollinators like the honeybee will be exposed to higher concentrations. Thus, higher proportions of 'disoriented' foragers could decrease foraging efficiency, leading to a reduction in the honeybee population. Such effects have been seen in neonicotinoid treatments (Henry et al., 2012; Schneider et al., 2012; Fischer et al., 2014). Schneider and co-workers (2012) recorded a significant reduction in the number of honeybees visiting the food source and returning to the hive after the exposure to imidacloprid and clothianidin. Moreover, bees spent longer periods inside the hive before restarting the foraging process to the food source. As a consequence of this impairment, the foraging efficiency of the colony as a whole might be affected.

The concentrations of herbicide used in our study were based on recommended levels for spraying fields and levels measured in natural environments (0 to 3.7 mg l^{-1} range; Couture et al., 1995; Mann and Bidwell, 1999; Giesy et al., 2000; Perkins et al., 2000; Solomon and Thompson, 2003), even though higher concentrations have not been previously measured in the environment, they were selected to represent a potential worst-case exposure scenario that a pollinator could encounter while foraging in flowers located within or outside the GM crops (Chang et al., 2011). Interestingly, the locomotive activity of bees tested in our study was not impaired after the incubation phase and they did not reject the sucrose solution offered, whether with or without GLY. As a result, honeybees continued foraging at our feeding station and thus also on plants that expose bees to similar GLY concentrations, and the contaminated nectar or pollen could be brought back by honeybees to the hive and would then accumulate there. Rubio and co-workers (2014) found traces of glyphosate in both organic (26–93 ppb, mean 50 ppb) and non-organic (17–163 ppb, mean 66 ppb) honey samples from several countries. Moreover, they found the presence of GLY traces in honey samples made by bees feeding on wild and melliferous flora. Although the amounts they reported are lower than the GLY concentration that we used in this study, it does not mean that this was representative of those concentrations the forager bees are exposed to in the field. We expect that some bees could find the concentrations of GLY that we used in our experiment in food and would then take it back to the hive. With this in mind, further studies

Table 2. Data for control and GLY-treated bees released for the second time

GLY treatment	No. of bees released	No. arrived at hive	No. not arrived
0 mg l^{-1}	19	16 (0.84)	3 (0.16)
2.5 mg l^{-1}	11	7 (0.64)	4 (0.36)
5 mg l^{-1}	10	10 (1)	0 (0)
10 mg l^{-1}	4	4 (1)	0 (0)

Numbers in parentheses indicate the proportion of bees for each treatment.

in GLY-exposed commercial crops and their surroundings are necessary to evaluate the actual exposure of forager bees to the herbicide, and the relationship between the concentration of GLY collected by the honeybees in exposed environments and the traces found in the stored honey or pollen.

Despite the lack of data on the actual level of GLY that forager honeybees are exposed to in the field, present results show that exposure to non-lethal concentrations of glyphosate causes sub-lethal effects, which modify the bees' foraging behavior. However, further studies are necessary to evaluate to what extent this chemical influences foraging behavior of honeybees in a natural environment and whether prolonged exposure to this herbicide might contribute to worsen the health status of beehives. Since GM herbicide-tolerant crop fields are usually surrounded by native flora that is visited by honeybees, it would also be necessary to analyze traces of glyphosate present in collected and stored honey and pollen, as well as in larvae and adult bees from hives located in the surroundings of agricultural crops treated with GLY, before and after the herbicide application.

MATERIALS AND METHODS

Animals and study site

We used bees from a colony of approximately 30,000 bees (*Apis mellifera* Linnaeus 1758). The experiment was conducted from August to September of 2013 in an open field (N 50°48′53.01″, E 8°52′21.36″) located close to the village of Großseelheim (Hessen), Germany.

Experimental procedure

A group of forager bees was trained to collect unscented 0.5 mol l^{-1} sucrose solution from an artificial feeder located 400 m north of the hive and fitted with colored number tags on the thorax for individual identification. At 15 min intervals, numbered bees were captured individually at the feeder before they began to ingest the sucrose solution offered and were immediately confined in plastic tubes, and transported to the release site (RS) located 460 m east of the feeder location. The RS was located within the area explored during orientation flights, but otherwise it was novel for the trained bees. Each plastic tube contained a small feeder providing 50 µl of unscented 2 mol l^{-1} sucrose solution, either with or without glyphosate. The tube was kept in a dark box for 1 hour (incubation), allowing bees to ingest all the solution offered. Three different concentrations of GLY were used (diluted in 2 mol l^{-1} sucrose solution, see next section for more details): 2.5 mg l^{-1}, 5 mg l^{-1} and 10 mg l^{-1}. Control bees were handled in the same way but were fed the solution without herbicide (0 mg l^{-1}).

After incubation, a radar transponder was glued to the number tag fixed on the thorax of each bee and the homeward flight trajectory (from the RS to the hive) was tracked with harmonic radar. Bees were released at 15 min intervals to ensure the same incubation time for all the individuals. One experimenter at the radar station passed on information about the flight trajectories of the released bees by walkie-talkie. Once the bee arrived at the hive, it was caught, the transponder was removed, and then the honeybee was allowed to enter the hive. Whenever possible, these bees were captured at the feeder and released from the RS once more ('second release') in order to test whether learning during homing flights was compromised. The total number of bees tested was 108 for the first release, and 44 for the second. The number of flight trajectories obtained was 79 for first release and 37 for the second (see Tables 1 and 2).

We measured the following variables: capture time, release time, arrival time at the hive and the flight trajectory recorded with the harmonic radar. If a bee was observed on the radar but then disappeared from the radar range and was not seen arriving at the hive, it was classified as a non-arriving bee.

Herbicide

A stock solution of glyphosate (Glyphosate PESTANAL, Sigma-Aldrich, Steinheim, Germany) at a concentration of 100 mg acid equivalent l^{-1} was prepared with distilled water and kept refrigerated. New stock solution was prepared every 7 days. The stock solution was diluted in sucrose solution 2 mol l^{-1} to obtain the different GLY concentrations used in the experimental procedure. The concentrations of herbicide used were: 0 mg (control), 2.5 mg, 5 mg and 10 mg of glyphosate per liter of sucrose solution. Each bee ingested 50 µl of 2 mol l^{-1} sucrose solution with or without GLY, so the concentrations used were equivalent to the following doses: 0 ng, 125 ng, 250 ng and 500 ng of glyphosate per bee.

Harmonic radar tracking

Tracking bees with a harmonic radar system is described in Riley et al. (1996, 2005), Menzel et al. (2011) and Scheiner et al. (2013). We used a system with a sending unit which consisted of a 9.4 GHz radar transceiver (Raytheon Marine GmbH, Kiel, NSC 2525/7 XU) combined with a parabolic antenna of ~44 dBi that provided a signal from the transponder on the bee thorax every 3 s. The transponder consisted of a dipole antenna with a Low Barrier Schottky Diode HSCH-5340 of centered inductivity. The second harmonic component of the signal (18.8 GHz) was the target for the radar. The receiving unit consisted of an 18.8 GHz parabolic antenna with a low-noise preamplifier directly coupled to a mixer (18.8 GHz oscillator) and a downstream amplifier with a 90 MHz ZF-Filter. The transponder was made of a silver wire with a diameter of 0.3 mm, a length of 11 mm, a weight of 10.5 mg and a loop inductance of 1.3 nH. The range of the harmonic radar had a radius of 900 m.

Statistical analysis

A heterogeneity chi-square analysis was used to compare the proportion of bees performing direct or indirect flights from the release site back to the hive. A Kruskal–Wallis test was performed to compare the time bees spent between the RS and the hive, according to the treatments (control bees and bees exposed to GLY: 2.5 mg l^{-1}, 5 mg l^{-1} and 10 mg l^{-1}). To compare the proportion of bees performing direct or indirect flights according to whether bees were released once or twice, we applied Fisher's exact test (Sokal and Rohlf, 1995).

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Competing interests

The authors declare no competing or financial interests.

Author contributions

M.S.B., R.M. and W.M.F. conceived and designed the experiments. M.S.B., U.G., M.-L.H., L.T. and R.M. performed the experiments. M.S.B., R.M. and W.M.F. performed data analysis. M.S.B., R.M. and W.M.F. drafted the manuscript. All authors revised and commented on the manuscript.

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Effects of Glyphosate and 2,4-D on Earthworms (*Eisenia foetida*) in Laboratory Tests

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Abstract Laboratory tests were conducted to compare the effects of various concentrations of glyphosate and 2,4-D on earthworms (*Eisenia foetida*) cultured in Argissol during 56 days of incubation. The effects on earthworm growth, survival, and reproduction rates were verified for different exposure times. Earthworms kept in glyphosate-treated soil were classified as alive in all evaluations, but showed gradual and significant reduction in mean weight (50%) at all test concentrations. For 2,4-D, 100% mortality was observed in soil treated with 500 and 1,000 mg/kg. At 14 days, 30%–40% mortality levels were observed in all other concentrations. No cocoons or juveniles were found in soil treated with either herbicide. Glyphosate and 2,4-D demonstrated severe effects on the development and reproduction of *Eisenia foetida* in laboratory tests in the range of test concentrations.

Keywords Terrestrial ecotoxicology · Earthworm · *Eisenia foetida* · Contaminated soils · Herbicides

Earthworms are important members of the soil fauna and possess a number of characteristics that make them appropriate organisms for use in assessing potential risks of contaminated soils. Earthworms are affected by a variety of organic and inorganic compounds, which may cause bioaccumulation. They are also important in the terrestrial trophic system, constituting a food source for a wide variety of organisms, including birds, mammals, reptiles, amphibians, fish, insects, and microorganisms.

Eisenia foetida has been chosen as a test species because: (1) it reproduces easily in the laboratory; (2) it is the most widely used species in laboratory experiments; (3) it was approved by the European Union and OECD for use in toxicity tests; and (4) it has been used by the U.S. Environmental Protection Agency (EPA) as a sweeping test for contaminant residues in several polluted sites.

Toxicity studies with earthworms are still scarce in Brazil. Preliminary results indicate that the behavioral test can serve as a rapid indicator of the toxicity of contaminated soils and can be used as a complementary test for risk assessment of polluted areas (Sisino et al. 2006).

Pesticides are important pollutants of natural habitats. Among the pesticides used in Brazil, glyphosate and 2,4-D are the most widely used herbicides. Glyphosate is a broad-spectrum, post-emergence, non-selective aminophosphonate-type herbicide. In the early twenty-first century, glyphosate became the largest-selling single crop-protection chemical product on the market (Woodburn 2000). Glyphosate is generally regarded as an environmentally friendly herbicide due to its biodegradation and strong adsorption to soil (Barja & dos Santos Alfonso, 2005; Vereecken 2005). However, evidence from some experiments suggests that glyphosate reduces the growth of *A.caliginosa* when applied repeatedly to laboratory cultures at 2-week intervals, at a rate lower than commercially recommended (Springett and Gray 1992), but had no effect on *A. caliginosa* in another pot experiment where the chemical was mixed with soil (Martin 1982). Another study suggests that glyphosate, even at the recommended field dose, can cause cell death and interfere with non-specific esterase activity in the intestinal epithelial lining of

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P. elongate, causing at least 50% mortality in this worm population (Morowati 2000).

2,4-D is a widely used herbicide in Southern Brazil due to its low cost and good selectivity. This herbicide has poor biodegradability and has been detected frequently in water courses (Chingombe et al. 2006). However, this pesticide causes several metabolic alterations and tissue necrosis in non-target organisms, including important members of the food chain such as fish (Gallagher and Di Giulio 1991). On the other hand, some authors have found that the acute risk to earthworms from use of 2,4-D is low (Dalby et al. 1995). Earthworms may be exposed from either single or multiple applications of 2,4-D to a wide variety of crops, but in particular from its use on pasture and turf. A laboratory toxicity study using the dimethylamine salt of 2,4-D on *Eisenia foetida* reported a 14-day LC50 of 350-mg/kg soil. This test was conducted by mixing a concentration of test substances in soil (WHO 1997).

The concentrations used in this experiment had been treated previously as recommended agricultural rates with the commercial product. However, depending on the specific weed control and management, the herbicide effect may be intensified due to the increased dose and the number of applications.

The current study aimed to test the effects of glyphosate and 2,4-D, two pesticides extensively used in Brazilian agriculture, on the growth and survival of *Eisenia foetida*.

Materials and Methods

Glyphosate [N-(phosphonomethyl)glycine] purity 99.7% and 2,4-D [2,4-dichlorophenoxyacetic acid] purity 99% were obtained from SIGMA CHEMICAL and ACROS ORGANICS, respectively.

Soil samples used in the experiments were collected in the experimental area of Embrapa Agrobiologia, located in the municipal district of Seropédica (Rio de Janeiro) and classified as Argissol (EMBRAPA 1997). The source was selected as one of the most representative soils in Brazilian territory. The soil is used extensively in corn, soy, sugarcane, and other crops in tillage and non-tillage systems. The soil samples showed the following characteristics: 608 g/kg sand; 112 g/kg silt; 280 g/kg clay; 10.8 g/kg organic carbon; and pH 5.5.

The *Eisenia foetida* earthworms were obtained from the Minhocário Arborium (RJ). They were carefully transported to the laboratory and acclimatized for 4 weeks before any experiment, at 20°C ± 2, in bovine manure. All tests were performed using adult earthworms (age less than 2 months and clitella well developed) and with individual weight from 300 to 600 mg (ISO 11268-1 1993).

For the experiments, 10 earthworms were transferred to each container with 400 g of soil prepared by adding different concentrations of glyphosate and 2,4-D (dry weight basis). Four replicates were analyzed for each concentration and 4 control containers, prepared under identical conditions without the addition of the target pesticides. The concentrations used were 1; 10; 100; 500; 1,000 mg/kg. The containers were covered with paper-filter with holes to maintain aeration conditions during the 56 days of the test. Soil moisture was standardized at 60% of maximum water-holding capacity, and the samples were maintained at room temperature (20 ± 2°C) in the presence of light. During all the experiments, moisture content was checked and maintained at 60% by adjusting the weight of the container against the weight known from the previous week prior to sampling. After 14, 21, 28, 42, and 56 days of incubation, the containers were opened, surviving and dead earthworms were counted, and the survivors' average weight was verified. Earthworms were classified as dead when they did not respond to a gentile mechanical stimulus and morphological abnormalities were recorded. For the reproduction test, cocoon production and numbers of hatched juveniles were hand-sorted and returned and further incubated during the experiment (ISO 11268-2 1998).

All the data were analyzed by ANOVA using Tukey's test for comparing the treatments and duration of exposure for each herbicide and control groups. The results were expressed as means ± SD (standard deviation). Statistical significance was set at $p < 0.05$.

Results and Discussion

The two herbicides showed different effects on the earthworms. In the growth test, earthworms from the soil treated with glyphosate showed a gradual reduction in mean weight during the experiment. The effect was observed at all test concentrations when compared to untreated soil (Fig. 1). The percentage of weight loss at the end of the experiment was approximately 50% of baseline weight. All earthworms were classified as alive at all moments of sampling. The growth rates of untreated worms were considered non-significant during the 56 days of the experiments. The average biomass of untreated worms was the same. In contrast, worms from glyphosate-treated soil samples, in terms of time dependency, were significantly different ($p < 0.05$) from the controls, whereas no concentration dependency was observed with this chemical.

For 2,4D, 100% mortality was observed a few hours after exposure of those organisms in soil treated with 500 and 1,000 mg/kg. After 14 days, mortality was around

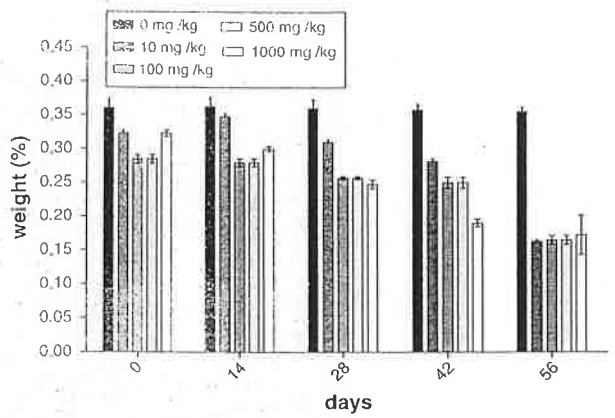

Fig. 1 Effect of Glyphosate soil concentration and exposure time on *Eisenia foetida* biomass

30%–40% of specimens in all the other concentrations. A gradual reduction in the mean weight of exposed worms (<30%) was also observed after 56 days in soils containing 2,4-D concentrations of 10 and 100 mg/kg, when compared to the soil controls (Fig. 2). The general tendency of these results showed that the decrease in weight was time- and concentration-dependent over the 56 days of exposure, which indicated statistical significance for both parameters ($p < 0.05$).

Comparing the results of the growth tests in soils treated with the two pesticides (glyphosate and 2,4-D), although glyphosate did not kill the test organisms in the range of test concentrations, the decrease in mean weight may indicate a chronic effect of this herbicide. In soils treated with 2,4-D, the observed effects (death of specimens after a few hours of exposure and loss of weight) are consistent with acute toxicity symptoms.

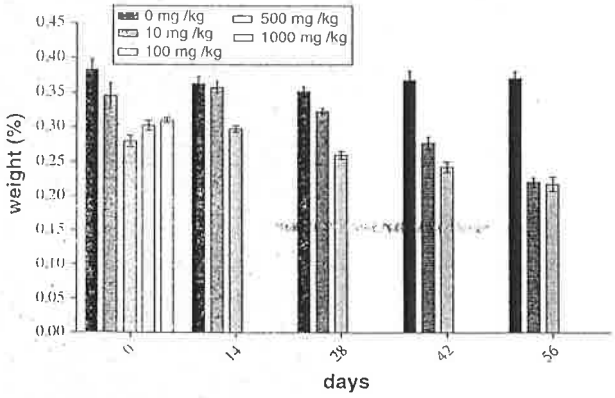

Fig. 2 Effect of 2,4-D soil concentration and exposure time on *Eisenia foetida* biomass

The reduction in mean weight of worms exposed to soils treated with 2,4-D (≤30%) when compared to those from soil treated with glyphosate (50%) can be explained by the morphological changes caused by the herbicides. In the presence of 2,4D, the emergence of abnormal swelling in the clitellar region of live worms increases their body volume and consequently their mean weight.

Significant anatomical changes were observed at 30 days (Fig. 3). Morphological abnormalities like elevating the body, coiling, and curling were observed in all specimens exposed to the highest concentrations of glyphosate and 2,4D after 30 days of exposure. Similar abnormalities were also observed in the organisms exposed for 50 days at lower concentrations. Excessive mucous secretion was noticed in all exposed worms. The exposed worms were also much less active than the controls. Approximately 65% of exposed worms displayed surface lesions and extrusion of coelomic fluid resulting in bloody lesions on the posterior part of the body and resulting in death. In soil treated with glyphosate, morphological changes (ruptures in the body wall and bloody lesions) were also observed in the midsection of the earthworm bodies (Fig. 3a–c). Fragmentation of the body was also observed as a result of these changes. Fifty percent of all treated worms developed breakages and shedding of cuticle (dried), which resembles ecdysis (shedding of skin) in insects and snakes (Fig. 3c).

In soil treated with 2,4-D, about 80% of exposed worms developed constrictions and swelling in the clitellar region (Fig. 3e, f), and most of the worms displayed multiple ruptures in the rectal region, with collapsed annular segment (Fig. 3d).

Table 1 shows the results of the reproduction tests in the soils treated with glyphosate and 2,4-D. No cocoons or juveniles were found in any experiment using soil containing the target herbicides. For both herbicides, reproduction was always lower than 20% when compared to the control, so that according to Dechema (1995), both can be classified as toxic. Despite the low reproduction observed in the controls, the absence of cocoons or juveniles in soils treated with both glyphosate and 2,4-D may result from interference by these substances in the earthworms' reproductive mechanism. The significant reduction ($p < 0.05$) in cocoons and juveniles was observed in all treated worms for both herbicides, compared to untreated worms. Additional studies are required to fully understand this effect. In addition, loss of weight was observed in the earthworms at the end of the experiment as compared to the controls, suggesting a significant toxic effect.

Soil ingestion and dermal absorption are the most important intake routes of soil pollutants by earthworms. In the present study, when the earthworms were introduced

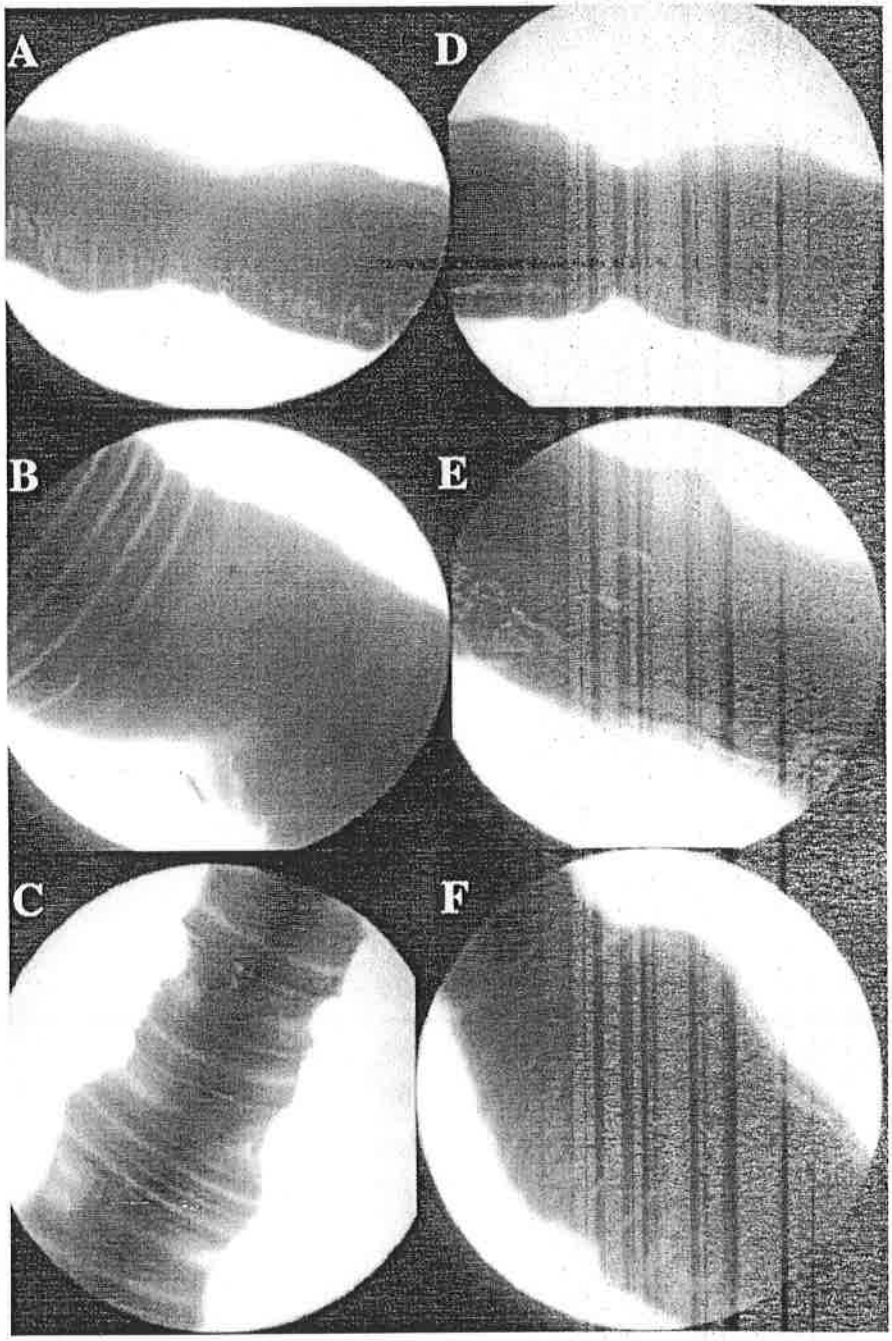

Fig. 3 Effect of glyphosate and 2,4-D on morphology of the earthworm, *E. foetida*, during 60 days of exposure. **a** Self-protection mechanism of anterior fragmented portion; **b** Loss of mucous and shedding of cuticular membrane; **c** Body constrictions and degeneration on posterior end; **d** Fragmentation on posterior region, attached to tiny portion of denatured body; **e** Coiling of earthworm along with abundant mucous; **f** Abnormal swelling on clitellar region and ruptures on body surface

into soils contaminated with 2,4-D, they remained on the surface, with death following after a few days (avoidance behavior). This behavior was not observed in soils treated with glyphosate.

Glyphosate and 2,4-D demonstrated severe effects on the development and reproduction of *Eisenia foetida* in laboratory tests. The toxic effects observed after exposure to soils contaminated with 2,4-D were much more severe than those observed with exposure to glyphosate at the same concentration. Meanwhile, long-term exposure (56 days) to soil contaminated with glyphosate demonstrated a toxic effect on normal development and reproduction of *Eisenia foetida*, indicating that this substance may have significant toxic effects on soil biota. A field study is being conducted at this laboratory to confirm these results in earthworm populations under natural conditions.

Table 1 Mortality (% of dead animals related to numbers of tested specimens) and reproduction test (total numbers of cocoons (28d) and hatched juveniles (56d))

Conc. mg/kg	Mortality (%)	Reproduction test		Conc. mg/kg	Mortality (%)	Reproduction test	
		Cocoons	Juveniles			Cocoons	Juveniles
Glyphosate				2,4-D			
0	0	7	4	0	0	5	2
10	0	0	0	10	30	0	0
100	0	0	0	100	40	0	0
500	0	0	0	500	100	0	0
1,000	0	0	0	1,000	100	0	0

These results were obtained from 4 replicates (n = 40 worms)

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Soil Association

The impact of glyphosate on soil health

The evidence to date

Glyphosate and Soil

Introduction

Over the last decade, about 6.1 billion kilograms of the herbicide glyphosate have been applied worldwide.[1] Glyphosate [N-(phosphonomethyl) glycine] is an active ingredient in a range of weed killer products, created for use in agriculture, horticulture and at amenity sites. Its use globally has risen almost 15-fold since 1996, when genetically engineered glyphosate-tolerant "Roundup Ready" crops were introduced.[2] In Great Britain in 2014, 1.9 million kilograms of glyphosate were used on agricultural and horticultural crops, on 2.2 million hectares.[3]

Despite being the most heavily applied herbicide in the world,[4] in 2015 glyphosate was classified as 'probably carcinogenic to humans' by the International Agency for Research on Cancer, the specialized cancer agency of the World Health Organization, following a review of evidence from human exposure studies and in research on laboratory animals.[5] Furthermore, in 2016, scientists raised their concerns about the safety of glyphosate based herbicides and call for new investments in epidemiological studies, biomonitoring, and toxicology studies.[6]

Whilst the effects of glyphosate on human health are coming under scrutiny, scientists are now concerned about our insufficient knowledge of the *ecological* safety of glyphosate, the way it behaves in the natural environment, how it interacts with living organisms, and the pathways through which it is degraded.[7]

Glyphosate has been considered an environmentally safe herbicide because it is assumed to be inactivated quickly after spraying due to rapid sorption onto particles in the soil, and its fast degradation by microbes.[8] In addition, the mechanism by which it kills plants (inhibiting the shikimic acid metabolic pathway)[9] is thought to be unique to plants and some micro-organisms, including bacteria, algae and fungi, and thus theoretically not a threat to mammals.[10] However, evidence from several studies now shows that glyphosate-based herbicides, via multiple mechanisms, can adversely affect the biology of mammals. Furthermore, the half-life of glyphosate, which gives an indication of its persistence in the soil and water, is believed to be longer than previously thought.[11] Recent research suggests that the herbicide persists longer with the return of crop residues containing glyphosate to the soil.[12] There is evidence to suggest that glyphosate-based herbicides can adversely affect aquatic invertebrate ecology[13] and research has also shown a negative impact on amphibian larvae (tadpoles)[14] and earthworms.[15]

As well as the active ingredient of glyphosate, there is also concern about impacts of the adjuvants (other chemical substances that are added such as solvents and surfactants) in commercial glyphosate products, where different formulations have been found to have different levels of toxicity compared to pure glyphosate.[16]

The Soil Association has reviewed the science on the impact of glyphosate on soils and soil life. For the world's most widely sold weed-killer, we found surprisingly little research has been done. What research there is shows contrasting results, significant uncertainty and some evidence that glyphosate causes harm. More research is urgently needed.

What about the impact of glyphosate on soil and soil life?

Soils are the foundation of our food security and yet a recent global scientific assessment found that 33 per cent of land is degraded due to the erosion, salinization, compaction and acidification and chemical pollution of our soils.[17] This report reviews the published and peer-reviewed scientific evidence about the impact of glyphosate-based herbicides on soils, soil micro-organisms and soil fauna.

Sorption of glyphosate onto soil and the risk of leaching

The risk of environmental pollution through the leaching of pesticides out of soils into water bodies is affected by how strongly the compound is sorbed to soil. (Sorption is the process by which one substance becomes attached to another and includes both adsorption and absorption). Compared to other pesticides, glyphosate is recorded to have strong sorption characteristics, reducing the risk of leaching. However, several studies have indicated that in some circumstances there is a risk of the leaching of glyphosate into deeper soil layers, where it could end up in ground and surface waters.[18] The level of sorption depends on several soil characteristics including mineral content and type, pH, soil redox conditions, phosphate content (that can compete with glyphosate for sorption sites) and possibly soil organic matter.[19] Rainfall and poor state of soils can increase the risk of glyphosate loss out of soils through erosion.[20]

Degradation of glyphosate

Micro-organisms in soil (bacteria and fungi)[21] are responsible for the degradation of glyphosate through two chemical pathways. One pathway produces a compound known as AMPA (aminomethylphosphonic acid) which is found in soils treated with glyphosate. This is thought to be mildly toxic to plant growth. The second pathway produces the compound sarcosine. The micro-organisms responsible for the degradation use enyzmes to break down glyphosate, to obtain a source of phosphorus, nitrogen and carbon for themselves.[22] Studies examining the rate of glyphosate degradation showed some variability in results, and the process can depend on a range of factors. There is some evidence for the rate of degradation being correlated with the population size of bacteria in the soils.[23] Overall, sorption of glyphosate onto soil particles is thought to decrease degradation, but glyphosate that has been sorbed can still be degraded by micro-organisms. Rates will vary with topographical features that effect water availability,[24] soil type, and increase with temperature.[25]

Effect of glyphosate on soil micro-organisms

Micro-organisms are a major portion of the biodiversity and biomass of soils and play a key role in maintaining soil processes, and thus the functioning of ecosystems. They are considered 'crucial to life', and are present in very large numbers.[26] 'Ecosystem services' is a term to measure the (monetary) 'benefits provided by ecosystems that contribute to making human life both possible and worth living'.[27] Microbial communities in the soil form the basis of ecosystem services such as the transformation of pollutants and the nutrient cycling[28] and underpin all provisioning and regulating services.[29] Several groups of scientists working in the field are calling for further research on the impact of glyphosate on microbial soil communities given the critical role that these organisms play in ecosystem services, including in maintaining plant health in agricultural systems.[30]

To date, scientific studies about the impact of glyphosate on soil micro-organisms have provided contrasting results. Some soil-based studies have not found any threat to soil micro-organisms from glyphosate.[31] Indeed, it is understood that glyphosate increases soil microbial activity when the herbicide is added because microbes break it down and use it as a source of carbon, nitrogen or phosphorus (as discussed above).[32] However, this is thought to be a short-term effect only.[33] One study found no effect on bacteria numbers from the use of glyphosate, but fungi and Actinomycetes (bacterium) numbers increased.[34]

In the forestry context, it has been found that in ponderosa pine plantations glyphosate has no consequential effects on soil communities in soil based tests.[35] Another study found a benign effect on microbial community structure when the commercial formulation of glyphosate was applied to soil samples at the recommended field rate, and produces a non-specific, short-term stimulation of bacteria at a high concentration.[36] A further study found that glyphosate has only small and transient effects on soil microbial community structure, function and activity on field scale experiments in agricultural soils.[37]

However, the potential non-target effects of glyphosate on soil micro-organisms are still of much concern amongst scientists.[38] There is concern about the diverse effects now reported about the impact of glyphosate on the biology and ecology of rhizosphere micro-organisms, and on their interactions with plant roots when released into the rhizosphere, the region of soil that surrounds, and is influenced by, plant roots.[39]

Effects on soil microbial community population, function and structure

A recent study suggested that glyphosate may have an indirect effect on the soil microbial community function and structure in arable ecosystems which should be further evaluated. This research, that looked at the impact of glyphosate (as RoundUp) on the soil bacterial communities in the rhizosphere of glyphosate-treated barley, found that the abundance of the culturable bacterial community, and the total bacterial composition were affected, and there was a proliferation of protists (a varied group of single celled organisms). This is likely due, at least in part, to an increased availability of easily degradable carbon compounds from the roots killed by the glyphosate.[40]

Another study examining the effects of glyphosate (as RoundUp) on soil bacteria, found that proteobacteria increased in relative abundance for corn and soya crops, following glyphosate exposure, whilst the relative abundance of Acidobacteria decreased in response to glyphosate exposure. This is a significant observation because Acidobacteria are believed to be highly involved in biogeochemical processes, such as for cellulose degradation. Decreases in the abundance of these bacteria over the long-term could impair the ability of soil to perform certain biogeochemical reactions performed by these organisms.[41]

Recent research demonstrated that a Roundup formulation (R450) was toxic to the soil fungus *Aspergillus nidulans*, at doses far below the recommended agricultural application rate, and concluded that the herbicide might potentially impair agricultural soil ecosystems.[42]

Impact on mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) improve water access and soil minerals for plants, improve drought tolerance and help with resistance against pathogens. Recent research has found that glyphosate (and/or its metabolite AMPA) reduces the spore viability and root colonisation of AMF, and could reduce plant diversity.[43] Another study found a 40% reduction of mycorrhization after the application of Roundup in soils that had been amended with the mycorrhizal fungi, *Glomus mosseae*.[44]

Impact of repeated glyphosate applications

There is evidence now to suggest that repeated glyphosate applications can impact on soil microbial communities as they adapt to repeated glyphosate applications.[45] One study found that a single exposure to soils of glyphosate (technical grade) caused only minor changes to microbial community function or structure. However, in soils where there had been no previous application of glyphosate, microbial respiration increased in response to glyphosate exposure. This potentially reflects a stress response of species sensitive to glyphosate. In contrast, in soils that have been chronically exposed to glyphosate, the microbes did not have this response. This is most likely due to the gradual elimination of glyphosate sensitive species.[46]

Another study, found a negative impact of glyphosate (RoundUp) on non-pathogenic soil borne microfungi species in boreal forest soil, and suggested that repeated herbicide applications caused a shift in the fungal species towards those more resistant to exposure.[47] This effect was not found in a study looking at the impact of long-term glyphosate use on soils in Argentina on soil microbial communities. One possible reason for this lack of difference could be explained by the adsorption of glyphosate to soil which would make it unavailable for microbial communities.[48]

Increase in micro-organisms causing disease in crops

It has been reported that using glyphosate as a weed control in agricultural systems has led to the increased severity or re-emergence of crop diseases.[49] There is concern over how the use of glyphosate increases the potential for the development of pathogen levels that affect crop health, altering the communities of rhizosphere microbes involved in nutrient transformation, and shifting the balance between micro-organisms that are beneficial and detrimental to plant health.[50] For example, one study found that the disease severity and frequency of the soil borne fungus *Fusarium solani* f. sp. *Glycines*, the cause of Sudden Death Syndrome, in glyphosate-tolerant soya beans was higher after application of glyphosate compared to no herbicide application.[51]

There is now evidence to suggest that it is not just the direct disruption of the shikimic acid metabolic pathway which is responsible for the herbicidal properties of glyphosate. It is now believed by some scientists that the herbicidal efficacy of glyphosate is largely due to colonization of roots of affected plants by soil-borne pathogens and that glyphosate somehow compromises the ability of plants to defend against pathogens that inhabit the rhizosphere. Many of plants defences are reliant on the shikimic acid pathway, and as glyphosate blocks this pathway, it is conceivable that glyphosate would render plants more susceptible to pathogens.[52]

Research on glyphosate-resistant soybeans found that glyphosate altered particular rhizosphere micro-organisms.[53] In one study, the colonisation of roots by Fusarium fungi increased steadily as soybean growth progressed and as the rate of glyphosate increased. This suggests that glyphosate affects the ability of plants to suppress potential pathogen colonisation and root infection. Further, by suppressing Fluorescent pseudomonads bacteria and Mn-reducing rhizobacteria, glyphosate lowers two plant defence mechanisms for warding off pathogens.[54] A different laboratory study, found no effect of glyphosate (Roundup) on Trichoderma or Gliocladium genera of fungi. However, both Fusarium and Pythium fungi genera populations increased proportionally to the increase in glyphosate concentrations, a concern given that both genera contain plant pathogens.[55]

Conversely, one study has looked at the impact of glyphosate, active ingredient and glyphosate commercial formulations, in laboratory tests, at field concentrations, on four types of entomopathogenic fungi – fungi that are understood to play a *positive role in controlling* pest insects in agricultural systems. They found that glyphosate active ingredient had no impact on the fungi, but that the glyphosate formulations (different brands of Roundup) did have a negative impact. The authors say that it is important that the impact of the supposedly inert ingredients in these formulations is further studied.[56]

Impact on soil fauna: Earthworms

Earthworms act as 'ecosystem engineers' by shredding plant litter, mineralising it and soil organic matter in their guts, and producing casts that enhance soil nutrient availability and promote plant productivity. Their burrowing enhances soil root penetration and water infiltration.[57]

Whilst at least two studies have not indicated any negative effects of glyphosate on earthworms,[58] at least six other studies found damaging effects of the herbicide. One study found that the earthworm *Eisenia fetida* avoids soil contaminated by the glyphosate based herbicide Groundclear, and this impact on locomotor activity could compromise the survival of the worms.[59] In another study, the number of hatched *Eisenia fetida* Andrei cocoons was significantly reduced in earthworms exposed to Roundup treated soils, and the number of juveniles was also significantly lower, indicating that glyphosate has a deleterious effect on the viability of cocoons. This study too found that the earthworms also avoided the soil treated with the herbicide. Earthworms have chemoreceptors and sensory turbercles and present a high sensitivity to chemicals in the soil.[60]

Another laboratory study on *Eisenia foetida* earthworms demonstrated severe effects on the development and reproduction caused by glyphosate (active ingredient only) at a range of concentrations, indicating that it may have significant toxic effects on soil biota. There was a decrease in the mean weight of the earthworms, and no cocoons or juveniles were found in the soil containing the herbicide.[61]

In a different earthworm greenhouse experiment, Roundup application initially stimulated surface casting activity of *Lumbricus terrestris* L. However the number of produced casts ceased dramatically about one week after herbicide application; cumulative cast mass produced by *L. terrestris* four weeks after herbicide application was reduced by 46% compared to the area not treated by herbicides. The activity of soil dwelling earthworms, *Aporrectodea caliginosa*, was not affected. The reproduction success of both earthworm species substantially decreased after herbicide application. The hatching rate of cocoons decreased from 43% to 17% for *L. terrestris* and from 71% to 32% for *A. caliginosa* when cocoons were collected from soil without herbicide or with herbicide treatment, respectively.[62]

Another study looked at the impact of Roundup on ecological interactions between the earthworm species, *Lumbricus terrestris*, and symbiotic arbuscular mycorrhizal fungi (AMF). The applications of Roundup reduced earthworm activity (as measured by the disturbance of toothpicks) in areas which contained AMF only. Earthworm activity (as measured by surface cast production) was not influenced by the application of Roundup or the presence of AMF. The application of Roundup led to earthworms that were heavier and that were less active at the surface. This is probably because there was abundant food in form of dead roots, or AMF in the soil that prohibited the earthworms from foraging from the surface.[63]

A recent 2016 study found that in the long term (132 days), the continuous consumption of leaf litter contaminated with glyphosate (Cheminova) decreased the earthworms (*Pontoscolex corethrurus*) growth rate, with a clear decline in their total biomass. However, no consistent effect was seen on cocoon production.[64]

Research into the glyphosate on other soil fauna is extremely limited. A meta-analysis of the impact of a range of herbicides on soil nematodes concluded that herbicides do affect soil webs with a variety of impacts on different nematode assemblages.[65] The only study looking specifically at glyphosate and nematodes made a comparison with 'conventional herbicide' and not with soil that did not receive any herbicide treatment.[66]

Further research required

The scientific evidence on the impact of glyphosate on the soil and soil life is far from conclusive. Research indicates potential impacts in increasing crop diseases, changing the composition and functioning of soil micro-organism species and ecosystems, and recently published studies are showing a negative impact on earthworms. Scientists working in this field are calling for future research to be carried out. This is urgent given the widespread and heavy use of glyphosate worldwide.

Recommendations for future research

- Research should examine the impact of glyphosate on other soil fauna in addition to earthworms; nematodes, ants, beetles, termites, spiders, anthropods, molluscs and protozoa.
- Research should look at both the impact on specific species of soil fauna and microflora but also the 'knock-on' succession effects of changes in the soil ecosystem.
- Research should consider the differences between the impact of glyphosate on soils as an active ingredient only, and when it is combined with other ingredients in a range of commercial products.
- Many of the studies described in this report have looked at glyphosate in the context of its use in relation to genetically-modified glyphosate resistant crops. In the UK context, where GM crops are not commercially grown, it is just as important to consider the use of glyphosate in relation to non-GM crops, and in amenity situations.
- Research should consider whether there is a significant build-up of AMPA in soils, which is produced when glyphosate is broken down, and whether this is problematic or not, as it is considered mildly toxic to plants.

Cover image: Wheat being sprayed with glyphosate immediately before harvest.

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Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations

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Herbicide use is increasing worldwide both in agriculture and private gardens. However, our knowledge of potential side-effects on non-target soil organisms, even on such eminent ones as earthworms, is still very scarce. In a greenhouse experiment, we assessed the impact of the most widely used glyphosate-based herbicide Roundup on two earthworm species with different feeding strategies. We demonstrate, that the surface casting activity of vertically burrowing earthworms (*Lumbricus terrestris*) almost ceased three weeks after herbicide application, while the activity of soil dwelling earthworms (*Aporrectodea caliginosa*) was not affected. Reproduction of the soil dwellers was reduced by 56% within three months after herbicide application. Herbicide application led to increased soil concentrations of nitrate by 1592% and phosphate by 127%, pointing to potential risks for nutrient leaching into streams, lakes, or groundwater aquifers. These sizeable herbicide-induced impacts on agroecosystems are particularly worrisome because these herbicides have been globally used for decades.

During the past 50 years the human population has more than doubled, while the productive arable area has increased only by 10%[1,2]. As a consequence, the intensity of agricultural production has increased dramatically including the use of pesticides. Among pesticides, glyphosate-based herbicides are most widely used - hardly available data state a global usage of about 650,000 tons[3] at sales worth about 6.5 billion US $ in 2010[4]. Glyphosate-based herbicides have been so widely used because they are very effective, acting non-selectively on plants by inhibiting the shikimic acid metabolic pathway found exclusively in plants and some microorganisms[5]. Hence, animals should theoretically not be affected by the application of glyphosate. Moreover, glyphosate is considered environmentally friendly due to its fast degradation[5] and strong adsorption to soil particles that should reduce leaching losses from the soil profile[6]. Nevertheless, evidence that glyphosate-based herbicides can harm non-target organisms, particularly amphibians[7,8], symbiotic mycorrhizal fungi or earthworms continues to mount[9,10].

Earthworms constitute a majority of soil faunal biomass in many temperate agroecosystems, with up to 1000 individuals and 300 g of biomass in each square meter of land[11]. They act as ecosystem engineers[12] by physically shredding plant litter, mineralizing it in their guts (along with soil organic matter), and enhancing soil nutrient availability through the production of up to 40 tons of casts per hectare annually[13] that can promote plant productivity[14–16]. Earthworm burrowing also enhances soil

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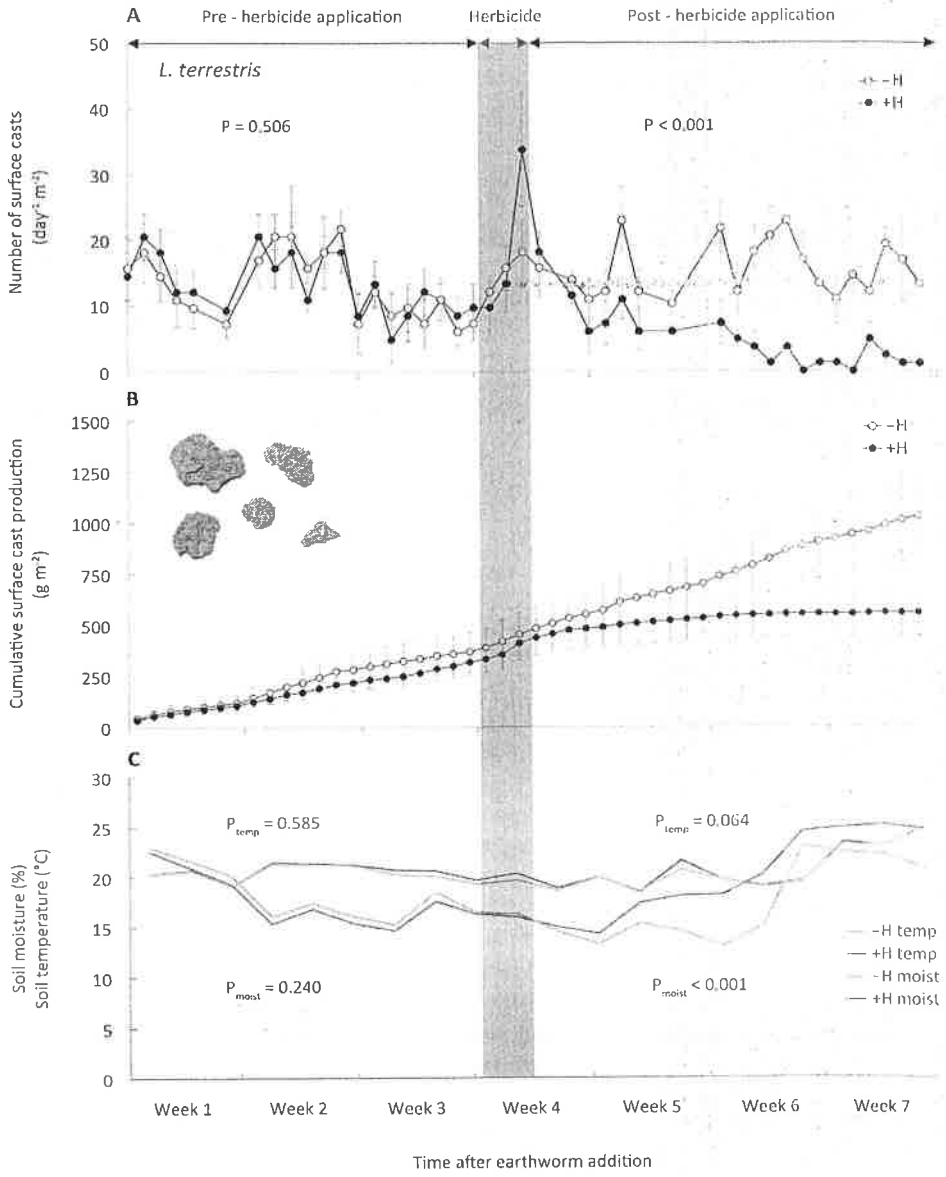

Figure 1. Activity of vertically burrowing earthworms before and after the herbicide application (-H, without herbicide; +H, with herbicide). (A) Daily surface cast production, (B) cumulative cast production over the course of the experiment, (C) time course of soil temperature (temp) and soil moisture (moist) (N = 6, mean ± SE). Red band marks period of herbicide application. P-values from two-sample Wilcoxon tests performed for the pre- and post-herbicide periods.

root penetration and water infiltration by constructing up to 8900 km of belowground channels per hectare[17]. Thus, earthworms strongly modulate agroecosystem function and services and any factor that may harm earthworms will impact ecosystem function, including plant growth and productivity[14,18,19].

Most studies that have examined the effects of glyphosate-based herbicides on the activity and reproduction of temperate earthworms have been conducted under laboratory conditions using compost worms (*Eisenia* species) that commonly do not inhabit agroecosystems[20–25]. Here, we present results of a greenhouse experiment testing the effects of a glyphosate-based herbicide on two earthworm species that are indeed frequently found in agroecosystems: the vertically burrowing anecic earthworm *Lumbricus terrestris* L. and the soil-dwelling endogeic species *Aporrectodea caliginosa* Savigny. We hypothesized that herbicide application would stimulate earthworm activity and reproduction due to the increased availability of dead plant material that earthworms can use as food source. As a knock-on effect, we expected that herbicide application via its effects on earthworms would also alter water infiltration, soil nutrient availability, and decomposition.

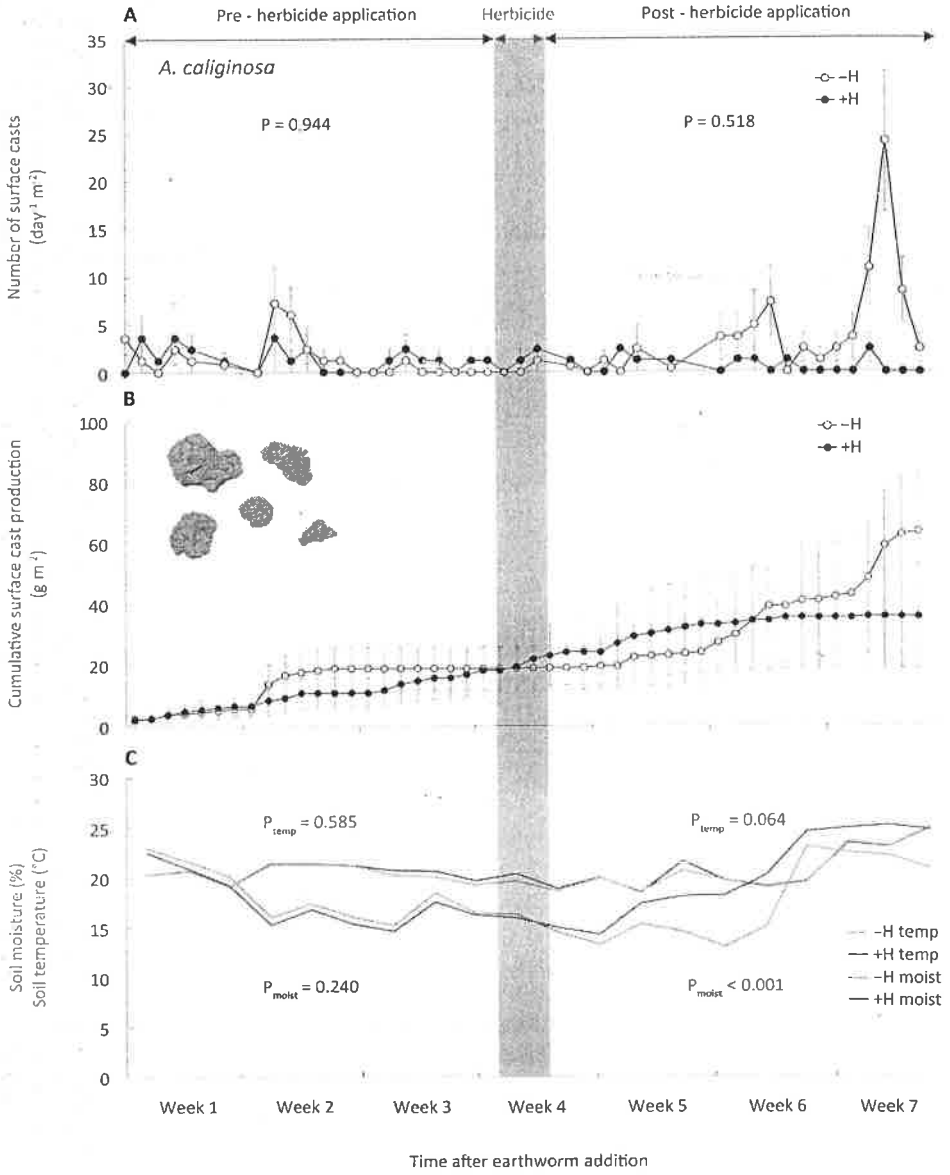

Figure 2. Activity of horizontally burrowing earthworms before and after the herbicide application (−H, without herbicide; +H, with herbicide). (A) Daily surface cast production, (B) cumulative cast production over the course of the experiment (N = 6, mean ± SE). Red band marks the period when herbicide was applied. P-values from two-sample Wilcoxon tests performed for the pre- and post-herbicide periods.

To test these hypotheses, we established weed communities comprising of a grass, a leguminous herb and a non-leguminous herb species commonly occurring in arable agroecosystems or garden beds. To these weed communities we added vertically burrowing or horizontally burrowing earthworm species. Eight weeks after planting, the vegetation in half of the mesocosms was treated with a lower-than-recommended dose of glyphosate-based herbicide.

Results and Discussion

Herbicide application initially stimulated surface casting activity of *L. terrestris*, however the number of produced casts ceased dramatically about one week after herbicide application; in contrast the surface casting activity of this species remained nearly constant when no herbicide was applied (Fig. 1A). Not only did exposure to herbicide reduce the number of surface casts produced, it also reduced the mean mass of individual casts (546 ± 202 mg cast^{-1} vs. 1,408 ± 140 mg cast^{-1}). Compared to non-herbicide treated mesocosms, cumulative cast mass produced by *L. terrestris* four weeks after herbicide application was reduced by 46% compared to untreated mesocosms (560 g m^{-2} vs. 1,032 g m^{-2}; $P < 0.001$; Fig. 1B).

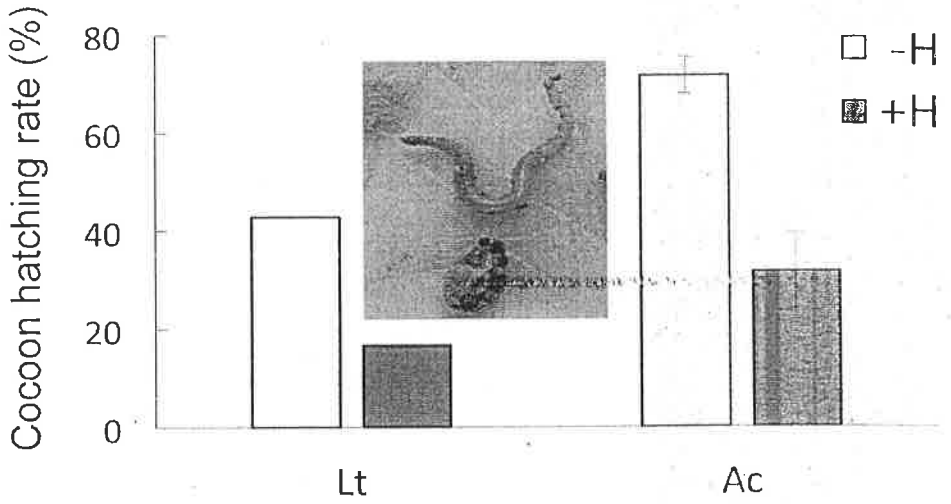

Figure 3. Percentage of cocoons with hatchlings of a vertically burrowing (*L. terrestris*, Lt) or a soil dwelling earthworm species (*A. caliginosa*, Ac) collected from mesocosms without (−H) and with (+H) herbicide application. (Lt: N = 1–2, Ac: N = 6, mean ± SE). Inset shows a cocoon with a freshly hatched *L. terrestris*.

Surface casting activity and cast mass production of the soil-dwelling earthworm species, *A. caliginosa*, was not affected by herbicide application (Fig. 2A,B). Monitoring surface casting activity has recently been proposed as an ecotoxicity test better related to earthworms' ecological role than standard laboratory tests[26]. Although the studied earthworm species differ in their feeding behavior, both have been shown to cast on the soil surface when foraging for leaf litter and other organic material[27,28]. The peak in surface casting activity observed after herbicide application was therefore likely the consequence of an increased availability of dead leaf material. Since we provided extra food for earthworms in all treatments (i.e., dried chopped hay spread over the soil surface) which is supposed to increase surface casting activity, the further decrease in casting activity in herbicide-treated mesocosms clearly demonstrates a direct impact of the herbicide. These detrimental effects of the herbicide on earthworm activity are also surprising as soil moisture increased between 3% to 39% after herbicide application (Fig. 1C) reflecting the lack of physiologically active, transpiring plants – however, increased soil moisture commonly stimulates casting activity[13,29,30]. Another explanation for the reduced surface casting activity after herbicide treatment might also be that *L. terrestris* avoided plant residues contaminated with glyphosate on the surface. As a consequence these earthworms might have lived in deeper soil horizons and avoided surface foraging and casting. This might also suggest the – albeit not significant – higher water infiltration in mesocosms with this species when exposed to the herbicides (see below). Overall, at the end of the experiment we retrieved 93.3 ± 6.6% and 86.7 ± 9.9% of introduced numbers of *L. terrestris* and 100.0 ± 0.1% and 100.0 ± 2.6% of introduced numbers of *A. caliginosa* in −H and +H treatments, respectively.

Reproduction success of both earthworm species substantially decreased after herbicide application. In total we found 25 cocoons from L. *terrestris* (18 cocoons in two −H, 7 cocoons in one +H mesocosm) and 292 cocoons from *A. caliginosa* (193 cocoons in six −H, 99 cocoons in six +H mesocosms). Hatching rate, i.e., percentage of cocoons from which earthworms hatched, decreased from 43% to 17% for *L. terrestris* (no statistical test was performed because of two few replications among treatments) and from 71% to 32% for *A. caliginosa* ($P < 0.001$) when cocoons were collected in mesocosms without herbicide or with herbicide treatment, respectively (Fig. 3). In ecotoxicological trials in the laboratory without plants glyphosate herbicide has also been shown to decrease the growth of *A. caliginosa*[31,32] and reproductive output of compost worms (*E. andrei* and *E. fetida*)[21,22]. However, to our knowledge, the current data are the first to demonstrate in a near-realistic setting side effects of glyphosate-based herbicides on the surface casting activity and reproduction of earthworm species that actually inhabit agroecosystems and will consequently come in contact with these pesticides.

Parameters indicating important ecosystems services were also affected by herbicide treatment. After herbicide application, all plants in our mesocosms were killed within a couple of days. As a consequence plant available nitrate in the soil increased by 1592% and plant available phosphate by 127% (Fig. 4A,B), probably attributable to a decrease in nitrate and phosphate uptake by plants[33]. While, no effect of glyphosate herbicides on soil decomposition rate was found, as in previous studies[21], the herbicide application tended to increase the stabilization factor of litter in soil suggesting a conversion from labile into more recalcitrant compounds (Fig. 4C; *26*). Herbicide application had no immediate effect

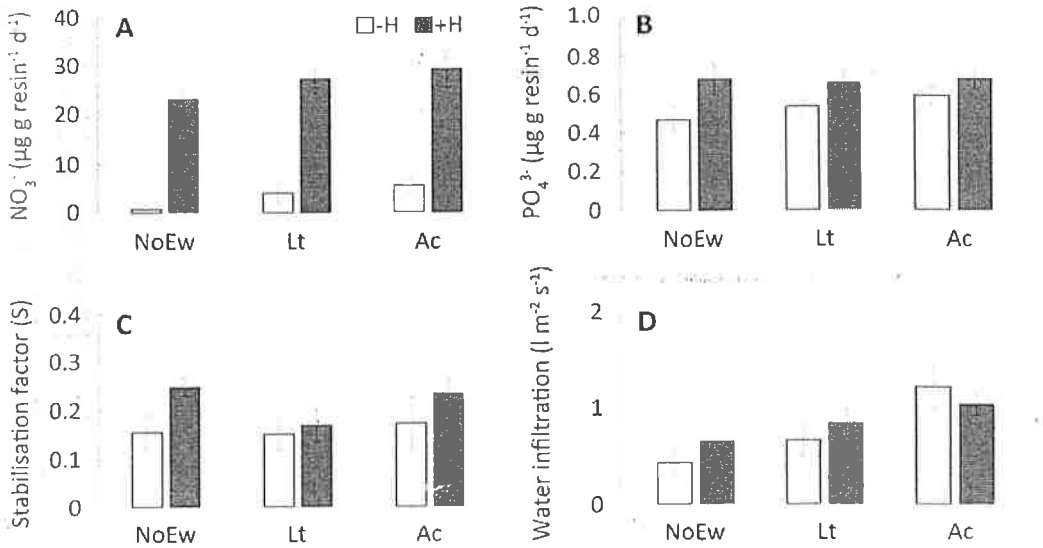

Figure 4. Soil parameters affected by herbicide application (–H, without herbicide; +H, with herbicide application) in response to the presence of different earthworms (NoEw, no earthworms; Lt, *L. terrestris*; Ac, *A. caliginosa*). (**A**) Plant available nitrate (NO^{3-}), (**B**) plant available phosphate (PO_4^{3-}), (**C**) soil stabilisation factor, and (**D**) water infiltration rate. (N = 6, mean ± SE).

on water infiltration after a simulated heavy rainfall event of 40 l m^{-2} (Fig. 4D). This was surprising as particularly vertically burrowing earthworms species such as *L. terrestris* are known to facilitate water infiltration[14,34]. However, the soil dwelling *A. caliginosa* that were less affected by herbicides in our study increased water infiltration (Fig. 4D, Table 1). These soil dwelling earthworms create short disconnected burrows with small diameters[35] and have also been found to increase water infiltration rates in other studies[14,36].

Because earthworms play a pivotal role in co-determining how agro- and garden ecosystems function, the observed deleterious side effects of glyphosate-based herbicide application indicate far-reaching consequences of its use in ecosystems. First, the role of earthworms as important ecological engineers in agroecosystems and gardens can be compromised[10]. Reductions in mixing of organic litter within the soil will limit long-term soil microbial activity[9], effects of earthworms on aboveground herbivores[37–39], soil nutrient cycling and availability, all of which may lead to reductions in plant productivity[40]. Second, pulses of nitrate and phosphate availability following herbicide application could increase the risk of leaching or surface runoff of these nutrients into groundwater systems or adjacent aquatic ecosystems, as long as the crop cover is not yet re-established. Obviously, official testing of potential side-effects during registration procedures failed to identify these ecologically important impacts[41]. Although productivity in many agricultural systems depends on the use of pesticides, findings from our study strongly indicate that more serious attention has to be paid testing pesticides for potential undesirable ecological side effects, especially in light of the projected doubling of global pesticide use by 2050[2].

Variable	Herbicide (H)		Earthworm (Ew)		H x Ew	
	F	P	F	P	F	P
NO_3^- (µg resin^{-1} day^{-1})	176.477	**<0.001**	3.404	**0.047**	0.062	0.940
PO_4^{3-} (µg resin^{-1} day^{-1})	6.827	**0.014**	0.426	0.657	0.490	0.617
k	2.297	0.140	0.249	0.781	1.392	0.264
S	3.789	0.061	0.946	0.400	0.528	0.595
Infiltration (l m^{-2} s^{-1})	0.401	0.532	7.247	**0.003**	1.041	0.366

Table 1. Summary of two-way ANOVA results testing the effects of herbicide application and earthworms on plant available nitrate (NO_3^-) and phosphate (PO_4^{3-}), decomposition rate (*k*), stabilization factor (*S*), and water infiltration rate. Significant effects in bold.

Methods

Study system and experimental setup. The experiment was performed between March and July 2013 in a greenhouse at the University of Natural Resources and Life Sciences Vienna (BOKU), Austria. We used 36 plastic pots (volume: 45 l, diameter: 42 cm, depth: 39 cm) filled with a 70 : 30 (vol/vol) mixture of soil from an arable field (soil type: Haplic Chernozem; BOKU Experimental Farm Groß-Enzersdorf) and quartz sand (grain size 1.4–2.2 mm) to create mesocosms. The substrate was homogenized using a concrete mixer, sieved (10 mm mesh size) and filled into the pots at a bulk density of 1.3 g cm^{-3}. The chosen mesh size might not completely retain juvenile earthworms or cocoons already present in the field soil; however because of the thorough mixing and random distribution of the substrate among the experimental pots homogeneity across treatments can be assumed. The substrate had the following characteristics: total C = 4.41 ± 0.06 mg g^{-1}, total N = 0.16 ± 0.01 mg g^{-1} (C:N ratio 27.6), K = 3.18 ± 0.12 mg g^{-1}, P = 0.62 ± 0.04 mg g^{-1}, and pH ($CaCl_2$) = 7.45 ± 0.02 (mean ± SE). To provide an initial food source for the endogeic earthworms (see below), 1.5 g dry mass of shredded grassland hay l^{-1} soil was added to all mesocosms.

The mesocosms were planted with three types of plant species: the grass *Dactylis glomerata* L., the leguminous herb *Trifolium repens* L., and the non-leguminous herb *Taraxacum officinale* F.H.Wigg. The three species are common weeds on agricultural fields (e.g. arable land, vineyards) across Central Europe. Plants were germinated from seeds obtained from a commercial supplier specialized in wild plant populations (Rieger-Hofmann GmbH, Blaufelden-Rabholdhausen, Germany). When seedlings were 1 cm high, 17 seedlings per species were transplanted to the pots in a triangular pattern (5.5 cm between plant individuals; plant density: 51 plants $mesocosms^{-1}$). During the experimental period, each mesocosm was irrigated equally using an automatic sprinkler system; mesocosms were placed on slats, allowing for free drainage.

Three weeks after planting, three earthworm treatments (n = 12) were established. The thirty-six mesocosms either received five specimens of adult vertically burrowing (anecic) *Lumbricus terrestris* L. (Lt) $mesocosm^{-1}$ (25.5 ± 0.7 g $mesocosm^{-1}$; ~183 g m^{-2}), ten adult/sub-adult specimens of the horizontally burrowing (endogeic) *Aporrectodea caliginosa* Savigny (Ac) $mesocosm^{-1}$ (12.09 ± 0.30 g $mesocosm^{-1}$; ~87 g m^{-2}), or no earthworms (NoEw). All earthworms were carefully rinsed, dried with filter paper and weighed before insertion; earthworm stockings for *L. terrestris* are in the upper range of natural abundance in temperate arable fields[42]. *A. caliginosa* was hand-collected from garden soil by one coauthor (JGZ) near the city of Eisenstadt (Burgenland, Austria), *L. terrestris* was purchased in a fishermen bait shop in Vienna. Earthworms were stored in boxes filled with the soil mixture for 5 days before transferred into mesocosms. All earthworms appeared to be in good health and buried themselves into the soil within a few minutes. Two times during the experiment 7.0 g of shredded hay were applied on the soil surface of each mesocosm, providing an additional food source. In order to prevent earthworms from escaping the mesocosm, drainage holes at the bottom of all pots were covered with garden weed fleece and the upper rim of all pots was extended with a 20 cm high, slightly outward bending barrier of transparent plastic film brushed with soft soap[39].

Eight weeks after planting, mature plants (*D. glomerata* was about 40 cm high, *T. repens* 19 cm, *T. officinale* 31 cm) of half of the mesocosms were treated with the herbicide 'Roundup®' (treatment +H), whereas the other half of the mesocosms remained untreated (treatment -H). Each +H mesocosm was sprayed with 7.2 ml of 'Roundup® Alphée' (glyphosate concentration 7.2 g l^{-1}; Scotts Celaflor, Mainz, Germany) on two consecutive days (in sum 14.4 ml), and 10 ml of 'Roundup® Speed' (glyphosate concentration 7.2 g l^{-1}; Scotts Celaflor, Mainz, Germany) two days afterwards. In total for all applications, 176.12 ml m^{-2} of herbicide was applied which is 53% lower than the recommended plant-based application rate of 1000 plants l^{-1} for 'Roundup® Speed' and 62% lower than the recommended dose of 800 plants l^{-1} for 'Roundup® Alphée' (Monsanto Co., St. Louis/Missouri, USA). The manufacturer recommends both herbicides to be used as an areal application prior to planting new lawns or garden beds, however it is unclear how often these products are actually applied together. Both products are sold in ready-to-use spray bottles - according to the manual we sprayed the products homogeneously to wet all weed leaves. The manufacturer suggests repeated treatments especially for perennial weeds (www.monsanto.com).

The treatments were replicated six times in a full-factorial design: three earthworm treatments (no earthworms, Lt, Ac) and two herbicide treatments (−H, +H). To encounter influences from microclimatic gradients, the mesocosms were placed in a randomized complete block design. Soil moisture in the upper 30 cm of each mesocosm was monitored using a TDR system (6050 × 1 Trase System I; Soil moisture Equipment Corp., Santa Barbara, CA, USA); soil temperature in each mesocosm was measured at 10 cm depth (Digital thermometer az-8851; Guangzhou Orimay Electronic Co, Guangzhou, China). Air temperature and relative humidity were monitored using tinytags (Gemini Data Loggers, West Sussex, UK) at 1.5 m above the floor. Environmental conditions during the experiment: daily air temperature 22.6 ± 2.3 °C, relative humidity 58.6 ± 5.8%, soil temperature 20.2 ± 1.2 °C, and soil moisture 18.1 ± 2.6 vol% (means ± SE).

Measurements and analyses. The measurement of earthworm activity started ten days after the introduction of earthworms by collecting freshly produced casts on the soil surface in the morning. Surface casts were collected 20 times before, three times during, and 20 times after the herbicide

application; each time the casts were counted, collected, dried (50 °C, 48 h) and weighed. Cast production was expressed as number of casts produced m^{-2} day^{-1}.

The water infiltration rate (1 m^{-2} s^{-1}) was measured two weeks after the final herbicide application by simulating a heavy rain shower of about 40 l m^{-2} (5.5 l $mesocosm^{-1}$)[14]. The time from pouring the water onto the soil surface until the last visible water disappeared into the soil was recorded.

Plant-available nutrients in the soil were measured using ion exchange resin bags (Amberlite IRN-150; Alfa Aesar, Karlsruhe, Germany;[43]). The resin bags (7 × 7 cm nylon mesh bags, 50 µm mesh width, containing 4.5 g resin) were stored in 2 M KCl and were rinsed in deionized water. Five days prior to herbicide application one resin bag was installed per mesocosm at 10 cm depth. Excavation took place 30 days after the last herbicide application to prevent resin saturation in the rather nutrient rich soil. Collected bags were quickly rinsed in deionized water to remove adhering soil and kept refrigerated until further analysis. The solution was analyzed for NH_4^+ and NO^{3-} using a xMark Microplate Absorbance Spectrophotometer (BIO-RAD, Philadelphia, PA, USA) and PO_4^{3-} using an EnSpire Multimode Plate Reader (Perkin Elmer, Walthalm, MA, USA;[44]). Plant available NH^{4+} was below detection limit and was therefore not reported.

Decomposition rate in the soil was determined using the Tea Bag Index (TBI,[45]). Therefore, two plastic tea bags containing either green tea (Lipton Unilever, USA: EAN 87 22700 05552 5) or Rooibos tea (Lipton: EAN 87 22700 18843 8) were buried at 10 cm depth in each mesocosm. Tea bags were removed 70 days after insertion (30 days after the last herbicide application). For calculating the TBI, consisting of the two parameters k (decomposition rate) and S (stabilization factor), the recommended calculated hydrolysable fractions (H; 0.842 g g^{-1} for green tea; 0.552 g g^{-1} for rooibos tea) were used[45]. During decomposition, parts of the labile compounds stabilize and become recalcitrant[46]. This stabilization depends on environmental factors[47] and results in a deviation of the actual decomposed fraction (i.e. limit value) from the hydrolysable (i.e. chemically labile) fraction. Stabilisation factor S is this deviation is interpreted as the inhibiting effect of environmental conditions on the decomposition of the labile fraction[45].

Destructive harvest of the mesocosms took place 32 days after the last herbicide application. Mesocosms were flipped over on a 2 × 2 mm mesh screen. In addition, a total number of 292 cocoons of *A. caliginosa* and totally 25 cocoons of *L. terrestris* were collected during the harvest. They were stored separated by treatment in plastic boxes (26 × 16.5 × 12 cm, length × width × depth, respectively) and mixed into the soil substrate used for the main experiment and kept in a dark basement (mean temperature 15 °C, >70% relative humidity). After 15 weeks, the number of hatched earthworms was counted and the hatchling ratio per treatment was calculated.

Statistical analysis. Residuals of all variables were tested for homogeneity of variances and normality using the tests after Levene and Shapiro-Wilk, respectively. Assumption for normality were not fulfilled by earthworm surface casting activity and associated soil temperature and moisture. Treatment effects for parameters not fulfilling the assumption for parametric tests were analyzed using the two-sample Wilcoxon test. Effects on decomposition, soil nutrients and water infiltration rate were measured either by one-way or two-way analysis of variance (ANOVA). Each significant ANOVA result ($P < 0.05$) was followed by Pairwise t tests as post-hoc comparisons with sequential Bonferroni corrections to account for differences in herbicide effects within earthworm treatments. All statistical analyses were performed using R (version 3.0.1; The R Foundation for Statistical Computing; http://www.R-project.org). Values given throughout the text are means ± SE.

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Author Contributions

M.G.B., B.R. and J.G.Z. conceived and planned the experiment; all authors jointly performed the experiment, analyzed the data and wrote the manuscript.

Additional Information

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Toxicity of AMPA to the earthworm *Eisenia andrei* Bouché, 1972 in tropical artificial soil

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Aminomethylphosphonic acid (AMPA) - one of glyphosate's main metabolites - has been classified as persistent in soils, raising concern regarding the widespread use of glyphosate in agriculture and forestry. Glyphosate may have negative or neutral effects on soil biota, but no information is available on the toxicity of AMPA to soil invertebrates. Therefore our aim was to study the effect of AMPA on mortality and reproduction of the earthworm species *Eisenia andrei* using standard soil ecotoxicological methods (ISO). Field-relevant concentrations of AMPA had no significant effects on mortality in acute or chronic assays. Except at the highest concentration tested, a significant biomass loss was observed compared to controls in the chronic assay. The number of juveniles and cocoons increased with higher concentrations of AMPA applied, but their mean weights decreased. This mass loss indicates higher sensitivity of juveniles than adults to AMPA. Our results suggest that earthworms coming from parents grown in contaminated soils may have reduced growth, limiting their beneficial roles in key soil ecosystem functions. Nevertheless, further research is needed to better understand the mechanisms underlying the sublethal effects observed here.

Glyphosate was first introduced in crop production in 1971[1]. However, its use expanded worldwide during the 1990's, when herbicide-resistant, genetically-engineered crops allowed widespread use by farmers of broad-spectrum herbicides such as glyphosate[2]. Genetically modified (GM) crops now cover 175 million hectares in 27 countries worldwide, but 77% of that area is located in only three countries: USA (40%), Brazil (23%) and Argentina (14%)[3]. The increased use of GM crops has been accompanied by a concomitant increase in glyphosate use. In Argentina, 238 million litres of glyphosate were sprayed in 2011, and in Brazil, approximately 340 million litres, raising concerns of the possible non-target effects of this herbicide, especially its potential impact on soil and water contamination and ecosystem functioning[4,5].

Earthworms are one of the most important components of the soil biota in terms of soil formation and maintenance of soil structure and fertility[6]. Furthermore, they are frequently used as indicators of soil quality and contamination levels, with standard, internationally recognized and adopted ecotoxicology assays[7–9]. These methods include acute and chronic tests. The former evaluates short-term and lethal effects of a potentially toxic agent, providing information on possible quick and dramatic changes in earthworm communities. On the other hand, chronic tests evaluate sub-lethal responses in longer-term parameters that are often more sensitive to soil pollution, such as growth and reproduction.

Although glyphosate effects on earthworms have been extensively studied, results are far from conclusive. Several studies consistently found very low mortality of *Eisenia andrei* Bouché, 1972 and *Aporrectodea* spp. worms at recommended (from 1,440 g ai.ha^{-1} to 1,800 g ai.ha^{-1}) and higher doses of glyphosate[10–16]. García-Torres *et al.*[17] reported significant mortality only at very high concentrations (50,000 mg.kg^{-1}), but Piola *et al.*[18] found that lethal doses depended on the commercial formulations used.

Conversely, sub-lethal parameters such as reproduction have usually been more sensitive than mortality in assessing glyphosate effects. For example, the number of juveniles and/or cocoons decreased at doses of 1 to 1,000 mg.kg^{-1}, 5,000 mg.kg^{-1} and 1,440 mg ai.ha^{-1} [11,12,17]. However, other studies found no significant effects of

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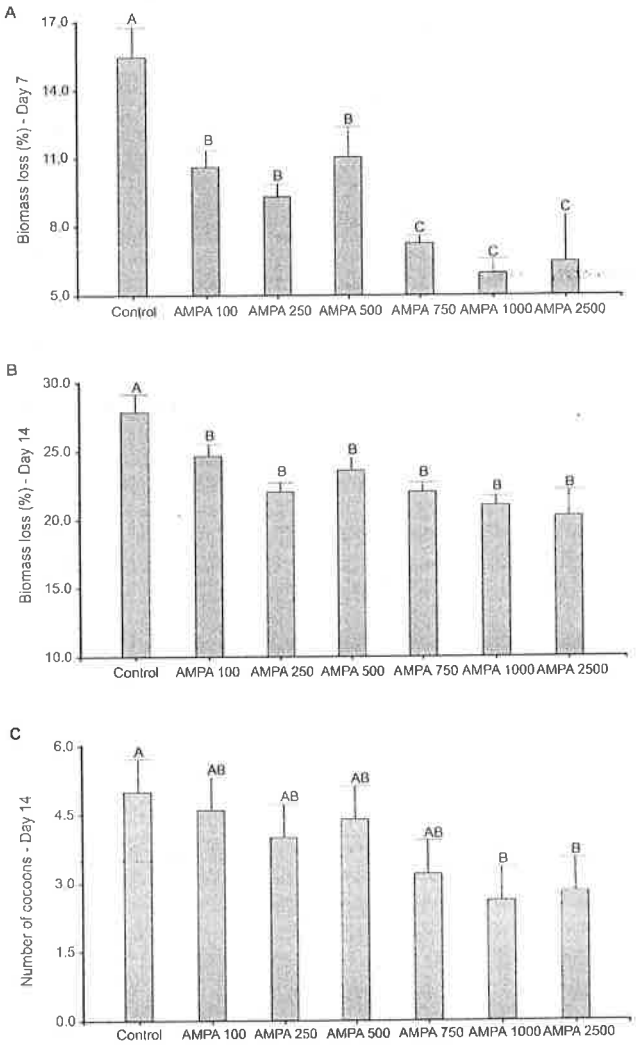

Figure 1. Effects of exposure to different doses of AMPA (100, 250, 500, 750, 1,000 or 2,500 $\mu g.kg^{-1}$) in tropical artificial soil on *Eisenia andrei* growth (presented as biomass loss, in % of initial weight) after 7 days (A) and 14 days (B) and on the number of cocoons (C), using the ISO (2012) standard acute toxicity test[8]. Different letters denote significant differences between treatments ($p < 0.05$).

glyphosate on earthworm reproduction[10,13,15,19]. Hence, the risk of non-target toxic effects of glyphosate in soils appears to be low, particularly considering its' short half-life: only 5 to 23 days[20–22] in field conditions, although the degradation pattern is significantly affected by factors such as soil texture, pH, rain events and microbial activity[23].

The major breakdown products of glyphosate are aminomethylphosphonic acid (AMPA) and sarcosine[20,24]. However, unlike glyphosate, AMPA has been classified as persistent in soils, with a typical half-life of 151 days, but varying from 76 to 240 days depending on field conditions[22]. The longer persistence of AMPA might result in higher toxicity risks compared to glyphosate, although very little information is available concerning AMPA toxicity[25–27]. To our knowledge, the effect of AMPA on soil invertebrates has not been previously studied. Therefore, the aim of this paper was to study the effect of AMPA on mortality and reproduction of the standard ecotoxicological test earthworm species *E. andrei*[8] in tropical artificial soil (TAS).

Results

Acute toxicity test. No significant mortality was observed in the control and in any of the tested AMPA concentrations. Only two earthworms died in the 500 $\mu g.kg^{-1}$ treatment. Biomass loss (Fig. 1A) at day 7 was lowest (5.98–7.26%) at the three highest doses (750 to 2500 $\mu g.kg^{-1}$), intermediate (9.32–11.05%) at the lowest doses (100 to 500 $\mu g.kg^{-1}$), and highest (15.41%) in the control. At day 14 (Fig. 1B) biomass loss was higher in the control (29.7%) than in all other treatments, and similar regardless of the AMPA dose applied. The higher mass loss in the control treatment was accompanied by energy and biomass investment in reproduction, as cocoon

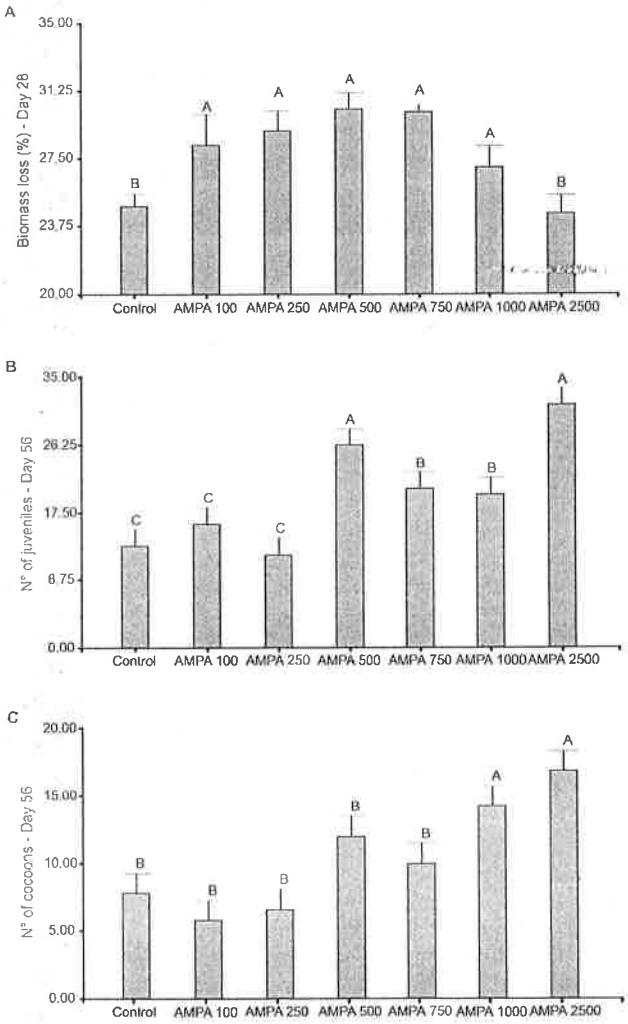

Figure 2. Effects of exposure to different doses of AMPA (100, 250, 500, 750, 1,000 or 2,500 $\mu g.kg^{-1}$) in tropical artificial soil on *Eisenia andrei* growth (presented as biomass loss, in % of initial weight) after 28 days (A), and on the number of juveniles (B) and cocoons (C) after 56 days, using the ISO (2012) standard chronic toxicity test[8]. Different letters denote significant differences between treatments ($p < 0.05$).

production was highest in the control and significantly higher when compared to the AMPA1000 and AMPA2500 treatments (Fig. 1C).

Chronic toxicity test. No significant mortality was found in control and in all other treatments, and only one earthworm died in the AMPA2500 treatment after 28 days. In contrast to the acute assay (above), biomass loss (Fig. 2A) was significantly lower in the control treatment compared to all the AMPA treatments, except for AMPA2500. In the control soil, 13.2 juveniles were found and 7.8 cocoons on average (Fig. 2B,C) after 56 days, with no significant difference from the values found in the AMPA100 and AMPA250 treatments. In all the higher AMPA doses, the number of juveniles was significantly higher than in the control, and at the highest AMPA doses (1,000 and 2,500 $\mu g\ kg^{-1}$) the number of cocoons was also higher. However, the mean weights of both juveniles and cocoons were significantly lower in AMPA2500 than in all other treatments (Fig. 3A,B).

Discussion

According to ISO guideline 11268-1[8] the results of the mortality assays are valid when the mortality of adult worms in the controls is $\leq 10\%$. This criterion was met in the present study (0% mortality). According to ISO guideline 11268-2[8] the results of the reproduction assays are valid if the percentage of mortality of the adults observed in the controls is $\leq 10\%$, if the coefficient of variance (CV) of reproduction in the control does not exceed 30%, and if the rate of production of juveniles is at least 30 per control container. The first two criteria were fulfilled but the minimum rate of 30 juveniles per control container was not met ($n = 13$ juveniles in the present study). Nevertheless, as the latter criterion is often not met in control soils/substrates[11,12], we considered the present results as valid and report them here.

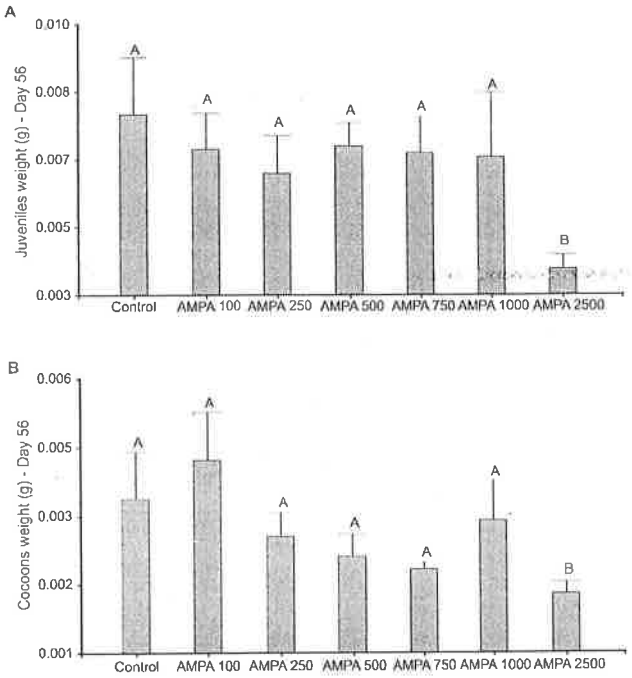

Figure 3. Effects of exposure to different doses of AMPA (100, 250, 500, 750, 1,000 or 2,500 $\mu g.kg^{-1}$) in tropical artificial soil on the fresh weight of *Eisenia andrei* juveniles (A) and cocoons (B). Different letters denote significant differences between treatments ($p < 0.05$).

The toxicity of the reference substance used (boric acid) was according to the expected. No cocoons or juveniles were produced in both the acute and chronic assays. In the long term assay, biomass loss was significantly higher compared with the control, as has been observed in other long term assessments[28]. Thus, the effect on reproduction was similar to that found by Becker *et al.*[29] at concentrations of 750 and 1,000 $mg.kg^{-1}$ boric acid. Therefore, both the sensitivity of the *E. andrei* specimens used and the laboratory test conditions were considered adequate, validating the present tests.

The absence of significant mortality in all the AMPA doses tested is consistent with most studies on glyphosate acute effects on *E. fetida* and *E. andrei*[10–13,17]. Therefore, field application rates and field concentrations of AMPA up to 2,500 $\mu g.kg^{-1}$ should not cause significant mortality of *E. andrei* in the short term (up to 28 days). Nevertheless, this does not discard the possibility of significant effects of AMPA on the survival of other earthworm species, since not all earthworm species respond in the same manner to soil contamination[5]. Nevertheless, it appears unlikely that there will be major effects on acute toxicity, considering the results already available for glyphosate and different earthworm species[10–13,17].

In fact, this is the first assessment of AMPA toxicity on a soil organism. AMPA acute toxicity has been described to be low for rats and moderate for fish and aquatic invertebrates[22]. In the fish *Anguilla anguilla* genotoxic damage was also shown[27], while in amphibians, the residence time in water was not significantly affected with doses up to 500 $\mu g.L^{-1}$ [30].

However, acute assays are generally less sensitive than chronic assays[31], which provide more realistic results on long-term sublethal effects, based on growth and reproduction. For instance, the acute assay did not reveal any negative effects of AMPA on biomass, and in fact earthworms in AMPA treatments after 7 and 14 days had lower biomass losses than earthworms in the control. On the other hand, at 28 days in the chronic test, biomass losses were significantly higher in all AMPA treatments (except the highest dose - AMPA2500) compared to the control.

The higher biomass loss in the acute test controls may be explained by the higher biomass investment in cocoon production in this treatment, particularly compared to the highest AMPA doses (1,000 and 2,500 $\mu g.kg^{-1}$). Another possible explanation could be that AMPA can be used as an extra source of food for microbiota[32] and could benefit earthworm growth. Indeed, high application rates of glyphosate have been found to stimulate microbial respiration[33–35], since glyphosate is a P-containing aminoacid that functions both as a sole P source for *in vitro* microbial growth and as a readily available C and N source when degraded in soil. Therefore, the same could be expected for AMPA. However, only very high glyphosate doses (5,000 $mg.kg^{-1}$) have been found as capable to significantly enhance microbial growth[32]. Moreover, our results tend to agree more with other studies that did not observe any significant effects of glyphosate on biomass loss, at concentrations of up to 200 $mg.kg^{-1}$ [16,14,19].

No significant mortality in the long term reproduction assay was found, which also agreed with the fore-mentioned studies on glyphosate toxicity to earthworms and on AMPA toxicity to other organisms. On the other hand, biomass losses in earthworms exposed for 28 days to AMPA doses from 100 to 1,000 $\mu g.kg^{-1}$ were significantly higher than in the control treatment. These results agree with Correia & Moreira[12] and Yasmin & Souza[16], who found negative effects of glyphosate at concentrations from 8 to 1000 $mg.kg^{-1}$ on earthworm

biomass, especially in the long term. However, there seems to be a threshold effect on earthworm growth, possibly somewhere in between the doses of 750 and 1,000 $\mu g.kg^{-1}$ AMPA, since at 1,000 $\mu g.kg^{-1}$ biomass loss was lower, and at the highest dose (2,500 $\mu g.kg^{-1}$) biomass loss was similar to the control and lower than all other AMPA doses. As observed in the acute trial, the biomass loss in the low to intermediate AMPA doses appear to be related to a higher investment in reproduction by the earthworms in these treatments. Long term exposure to AMPA to higher but non-lethal concentrations, seems to produce higher stress which is reflected both by an increase in cocoon production and by a higher biomass loss compared to the control.

Oxidative stress has been proposed as one possible mode of action of glyphosate in non-target organisms[36]. Activity changes of biotransformation system enzymes have also been used as indicators of sub-lethal impact of pollutants. In this sense, Contardo-Jara *et al.*[36] found an increase in soluble glutathione S-transferase (GST) in the blackworm *Lumbriculus variegatus*, at glyphosate doses ranging from 50 to 1,000 $\mu g.L^{-1}$. Interestingly, they also found a threshold between 1,000 $\mu g.L^{-1}$ and the highest dose tested: 5,000 $\mu g.L^{-1}$. Furthermore, they found no difference in soluble GST activity at 5,000 $\mu g.L^{-1}$ and the control treatment. Therefore, it is appears that the mechanisms by which AMPA exerts its toxicity on earthworms is different at high doses compared to intermediate and lower doses. Furthermore, the relationship between biomass loss and cocoon production appears to be different at the highest doses tested compared to the intermediate ones, suggesting that modes of action of AMPA at high doses are different for these two life-cycle parameters.

The number of juveniles found in the control soil after 56 days was greater than in Casabé *et al.*[11], Correia & Moreira[12] and García-Torres *et al.*[17], but lower than in Santos *et al.*[15]. Thus, the values we found were among those expected for *E. andrei* but with some variations compared to previous studies. The AMPA100 and AMPA250 treatments did not increase the number of juveniles compared to control, but at all higher doses, a significantly higher number of juveniles and a progressively higher number of cocoons were observed. A hormesis effect of glyphosate could be involved in these results. Hormesis is the phenomenon in which sub-harmful levels of a stress agent may help an organism in suboptimal environments[37]. In some cases, pesticides have been shown to increase total fecundity, fecundity dependent on dose, or advance fecundity to an earlier age[37]. Still, few studies have found a stimulation of cocoon production at low or intermediate doses of contaminants such as Pb, Al and Cu, which are toxic at higher doses[38]. For instance, in their review of pesticides effects on earthworms Yasmin & Souza[31] did not find any study reporting greater cocoon or juvenile production with increasing doses compared with controls. And while Gaupp-Berghausen *et al.*[39] found a substantial decrease in cocoon production of two earthworm species (*Lumbricus terrestris* and *Aporrectodea caliginosa*) due to herbicides with glyphosate as active ingredient, Santadino *et al.*[40] observed a significant increase in the number of *E. fetida* cocoons with increasing glyphosate doses. This result agrees with those of the present study and may be related to a hormesis effect. They observed lower fertility of those cocoons while we also found that the higher juvenile and cocoon production in the highest dose (AMPA2500) occurred together with a decrease in juvenile and cocoon biomass. Further studies including chemical and physiological evaluations will help reveal the mechanisms by which the higher stress produced by AMPA is transferred to a high juvenile and cocoon production, and how this is related to biomass loss.

The lower biomass found in cocoons and juveniles with higher AMPA doses might also be related to a higher sensitivity of juveniles than adults to AMPA, which would explain lower growth of juveniles, as has been previously observed[31]. Moreover, the production of more cocoons but lighter -and therefore presumably weaker- individuals, would be associated to weaker juveniles. This suggests that earthworms growing in soils contaminated with high doses of AMPA could have a lower physiological ability to develop, grow and reproduce in these soils and to accomplish key ecosystem functions.

Conclusions

In the present study, the toxicity of glyphosate's main metabolite – AMPA – to the earthworm *E. andrei* was studied in field-relevant concentrations. In both acute and chronic assays no significant mortality was recorded. Biomass loss in the short term assay was higher in the control compared to AMPA-contaminated treatments, probably due to energy and mass investment in higher cocoon production. However, in the long term assay, biomass loss was higher in AMPA treatments than in the control, except for the highest concentration in which a high production of significantly lighter cocoons and juveniles was recorded. This could be considered a reproduction disorder caused by AMPA. Hormesis is proposed as a possible mode of response of earthworms to AMPA, but further studies are needed to better understand the mechanisms and the physiology of AMPA toxicity to earthworms, and the role of low to intermediate contamination levels of AMPA in soils on earthworm growth and reproduction. This is especially important when considering the higher persistence of AMPA in soils compared to glyphosate, and the possible effects of long term exposure to high, but sub-lethal AMPA concentrations on earthworm populations and their potential roles in soil functioning.

Materials and Methods

Test species. *E. andrei*, the recommended species for ecotoxicological assessment with earthworms in guidelines for testing on chemicals[8,9], were purchased from Minhobox (Juiz de Fora, Minas Gerais, Brazil), an earthworm breeder who guarantees specimens free of any previous contamination. Before each assay, earthworms were maintained for 24 h in a box with uncontaminated substrate, to allow acclimatization to the laboratory conditions and to the artificial substrate. Only adult (with clitellum) specimens with 250 to 600 mg live weight and normal morphology, and responding to mechanical stimuli were used.

Test substrate. The assays were performed in tropical artificial soil (TAS), prepared according to García[41]. The TAS was a mixture of 70% fine sand, 20% kaolinite clay and 10% powdered coconut fibre, in replacement of sphagnum peat used in temperate artificial soils. Before mixing all the components, the sand was washed, air dried and sieved at 2 mm. Coconut fibre was also sieved (2 mm) and dried (60 °C). All components were mixed

until 40 kg of homogeneous substrate was obtained for all the assays (2 assays × 8 treatments × 5 replicates × 0.5 kg). Final pH was always within the optimal range determined by ISO guidelines, i.e., 6.0 ± 0.5, so adjustment with calcium carbonate was not necessary. Water holding capacity of the TAS was measured and moisture content maintained at 60% of water holding capacity during the entire period of each assay by monitoring weekly changes in individual vessel weights. The day before beginning each assay, the TAS was pre-moistened with deionized water corresponding to 30% of the water holding capacity. The remaining water was used to dilute the AMPA.

Test substance. AMPA (99% pure) was purchased from Sigma-Aldrich Co. (USA). The concentrations used in the assays were defined by field-relevant concentrations. To the best of our knowledge, 2,256 $\mu g.kg^{-1}$ is the highest environmental concentration of AMPA that has been reported in soils[4]. A 1 $mg.ml^{-1}$ aqueous AMPA stock solution was prepared using 10 mg of AMPA in 10 ml of water. This stock solution was used to obtain the nominal doses 100 (AMPA100), 250 (AMPA250), 500 (AMPA500), 750 (AMPA750), 1,000 (AMPA1000) and 2,500 (AMPA2500) $\mu g.kg^{-1}$. The spiking of the pre-moistened TAS at the desired doses was performed by dilution of AMPA in the deionized water volume equivalent to 30% of the water holding capacity. In addition, a negative control with water, and a positive control with a 945.94 $mg.kg^{-1}$ aqueous solution of boric acid[29] were prepared and incubated together with the spiked treatments. The use of a reference substance as positive control is indicated in the ISO guidelines since it permits evaluation of the sensitivity of test organisms used over time. In standardized ecotoxicological tests with earthworms, the fungicide carbendazim is the recommended substance. However, boric acid is also a suitable reference substance for *E.fetida/E.andrei*[7,28,29], so it was chosen for use in the present study. Boric acid causes a >50% reduction in abundance and biomass in field essays[28] as well as a marked reduction in biomass gain and in juvenile production in laboratory essays, at doses similar to the one used in this study[29].

Acute toxicity test assay. The acute toxicity test was performed according to the ISO 11268-1 guideline[8]. Five plastic vessels with a fine mesh in their lids to allow gaseous exchanges were filled with 500 g of the corresponding contaminated TAS for each one of the eight treatments. Each vessel received 10 earthworms, previously weighed and washed with deionized water. Each treatment had five replicates, totalling 400 specimens overall (8 treatments × 5 replicates × 10 individuals). The vessels were closed and kept in the dark at 20 ± 2 °C. After 7 days, dead earthworms were removed and the living individuals counted and weighed. All the specimens were again placed in the same vessels and after another 7 days (14 days total), dead earthworms were removed and the survivors counted and weighed for wet biomass determination.

Chronic toxicity test assay. The chronic toxicity test was performed according to the ISO 11268-2 guideline[8]. As in the previous assay, five vessels were filled with 500 g of the spiked TAS according to each of the eight treatments and 10 worms placed in each vessel. As a food source, 5 g of horse manure (oven dry at 60 °C) was placed on the surface of the substrate in each vessel. The vessels were closed and kept in the dark at 20 ± 2 °C. Throughout the test, water and food were checked weekly and added when necessary. After 28 days adult earthworms were carefully removed and the living ones counted and weighed. Soil was placed again in the same vessels and incubated for another 28 days. After 56 days, cocoons and juveniles were carefully hand-sorted, counted and weighed.

Data analyses. The biomass loss in the chronic and acute assays was expressed as the percentage of loss relative to initial weight, according to the formula: $100 - (W_x{*}100)/W_0$, where W_0 is the mean weight of the earthworms of each replicate (vessel) at the beginning of the assay and W_x is the mean weight of the earthworms found in each replicate at x days after the beginning of the assay.

A general linear model was used to evaluate the effect of the treatments on the assessed parameters (biomass loss, cocoon number and juvenile number). Error variance structure was modelled using treatment as grouping criteria and Var (Ident) of R's nlme library as variance function. When significant differences were found in the general model, *a posteriori* tests were performed by the DGC[42] or LSD tests. All statistical analyses were performed using the InfoStat software[43], as it is a friendly interpreter of R software.

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Author Contributions

A.D., G.G.B., K.D.S. and C.M.R.O. conceived the experiments. A.D. conducted the experiments. K.D.S. and C.M.R.O. helped in conducting the experiments. A.D. analysed the results. A.D., J.C.B., G.G.B. and C.C.N. discussed the data obtained. A.D., J.C.B., C.C.N., C.M.R.O., G.G.B., K.D.S., E.C.V. and M.L.C.B. provided

comments upon preparation of the manuscript. A.D. and J.C.B. wrote the paper. All authors reviewed the manuscript.

Additional Information

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752

Annexure XIV

Glyphosate Alternate Chemicals

Herbicide	Formulations	Approved for crop	Alternative herbicides registered
Glyphosate	Glyphosate 20.2% SL IPA salt	Non crop area	2,4-D Dimethyl Amine salt 58% SL 2,4-D Sodium salt Technical (having 2,4-D acid 80 % w/w) (Earlier Registered as 80%WP)
	Glyphosate Ammonium salt 20 % SL	Non crop area	2,4-D Dimethyl Amine salt 58% SL 2,4-D Sodium salt Technical (having 2,4-D acid 80 % w/w) (Earlier Registered as 80%WP)
	Glyphosate 41% SL (IPA Salt)	Tea	Oxyflourfen 23.5% EC Paraquat dichloride 24% SL Triasulfuron 20% WG
		Non-cropped area	2,4-D Dimethyl Amine salt 58% SL 2,4-D Sodium salt Technical (having 2,4-D acid 80 % w/w) (Earlier Registered as 80%WP)
	Glyphosate 54% SL (IPA Salt)	Non crop area	2,4-D Dimethyl Amine salt 58% SL 2,4-D Sodium salt Technical (having 2,4-D acid 80 % w/w) (Earlier Registered as 80%WP)
	Glyphosate Ammonium Salt 5% SL	Tea	Oxyflourfen 23.5% EC Paraquat dichloride 24% SL Triasulfuron 20% WG
		Non crop area	2,4-D Dimethyl Amine salt 58% SL 2,4-D Sodium salt Technical (having 2,4-D acid 80 % w/w) (Earlier Registered as 80%WP)
	Glyphosate 71% SG (Ammonium Salt)	Tea	Oxyflourfen 23.5% EC Paraquat dichloride 24% SL Triasulfuron 20% WG
		Non crop area	2,4-D Dimethyl Amine salt 58% SL 2,4-D Sodium salt Technical (having 2,4-D acid 80 % w/w) (Earlier Registered as 80%WP)