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भारत सरकार

Government of India

कृषि एवं किसान कल्याण मंत्रालय

Ministry of Agriculture & Farmers Welfare

कृषि एवं किसान कल्याण विभाग

Department of Agriculture & Farmers Welfare

वनस्पति संरक्षण, संगरोध एवं संग्रह निदेशालय

DIRECTORATE OF PLANT PROTECTION, QUARANTINE & STORAGE

केंद्रीय कीटनाशी बोर्ड एवम पंजीकरण समिति

Central Insecticides Board and Registration Committee

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N.H. IV, FARIDABAD (HARYANA)-121001

Dated: 14th September, 2022

PUBLIC NOTICE

Subject- Guidelines for registration of insecticides under section 9(3B), 9(3) and 9(4) -regarding.

The Registration Committee in its 440th meeting held on 03.08.2022 has approved the guidelines for registration of insecticides under section 9(3B), 9(3) and 9(4) vide minutes of 440th RC meeting (Agenda Item No. 10.58) which are enclosed for information of all stakeholders.

This has the approval of Secretary (CIB&RC).

Encl.: As above.

(Govind Ram)

Sr. Administrative Officer

Copy to:

1. Chairman, Registration Committee
2. PPS to JS (PP)/ PPS to PPA
3. IT Cell, HQ, Faridabad for uploading the same on the website.

Guidelines/Data requirement for grant of registration under various categories:

1. GUIDANCE TO STAKEHOLDERS-

- A. Stakeholders generating data and submitting the application for registration should ensure that the tests are conducted and data is generated in accordance with sound scientific procedures following the test guidelines and the principles of Good Laboratory Practice. The data should be authentic, of good quality and useful. The complete study reports should be submitted.
- B. The requirement for registration usually includes data and information on proposed application; data on identity of the insecticide (identity, composition, analysis and quality); data to assess risk to humans and the environment; data to assess efficacy of the product; and the packaging and labeling requirements.
- C. The data requirement for registration of insecticides varies with the type of insecticides to be registered (i.e. chemical or bio-pesticide and also type of bio-pesticide i.e. microbial pest control agent or botanical or semiochemical/pheromone); the type of material to be registered i.e. technical or formulation or Manufacturing Use Product (MUP); the type of formulation solid (WP, granules, powder) etc. liquid (EC, EW etc.) or vapour (vaporizer, fumigants etc.); the category of registration - i.e. provisional [u/s 9(3B)]; regular [u/s 9(3)] or subsequent 'Me-too' [u/s 9(4)] (Please refer section (9) of the Insecticides Act, 1968); purpose of registration - domestic use or export or for both domestic use and export; the intended use of the pesticide to be registered or its label claims etc. Hence, before starting data generation or submitting application for registration, the applicant should ensure that the requirements are being complied correctly for the type of pesticide to be

registered under the desired category and for the intended purpose.

- D. The data submitted by the applicant at the time of seeking registration under section 9 (3b) shall not be required to resubmit again at the time of submission of application for regular registration under section 9(3) by the same applicant, if chemical composition and other claims remain unchanged.

2. GUIDELINES FOR DATA REQUIREMENT FOR REGISTRATION OF PESTICIDES

These guidelines provide the guidance to the stakeholders to generate data and submit application with the indicated requirements. **The data requirements are divided into five segments, as envisaged below:**

- (A) Legal Requirements:** The documents required from legal discipline are at **Annexure I**.
- (B) The Data Requirements for Chemical Pesticides** are at **Annexure II**.
- (C) The Data Requirements for Microbial Pest Control Agents (MPCA) Pesticides** are at **Annexure III**.
- (D) The Data Requirements for Botanical/ Plant origin Pesticides** are at **Annexure IV**.
- (E) The Data Requirements for Pheromones/Semio-chemicals** are at **Annexure V**.

Note:

- 1. THE REQUIREMENTS/GUIDELINES FOR REGISTRATION OF INSECTICIDES EXCLUSIVELY FOR EXPORT REMAIN THE SAME AS APPROVED/RECOMMENDED BY THE REGISTRATION COMMITTEE IN ITS 380TH RC AND ARE AVAILABLE ON THE WEBSITE.**
- 2. The abbreviations used in the guidelines are in APPENDIX- I**
- 3. The terms used in the report are defined in APPENDIX- II**

LEGAL REQUIREMENTS

ANNEXURE-I

A. Legal Requirements:

1. Form - I duly signed/digitally signed
2. Copy of BOD Resolution/Affidavit/Partnership deed **(Notarized)**
3. Affidavit for Chemical composition on NJSP **(Notarized)**
4. Certificate as per category of Industry/ Manufacturing license **(Notarized)**
5. PAN No. **(Notarized)**
6. Incorporation Certificate **(Notarized)** and other documents as per KYC requirement
7. Proof of ownership/lease agreement of manufacturing site purported to be used as manufacturing site **(Notarized)**
8. Proof of Source of Technical to be used in formulation is duly registered. (In case of indigenous manufacture / import - Reference of RC meeting in which it was approved). {Only deemed registration status without issuance of Certificate of registration shall not be considered}.
9. Name of registrant of Formulation Import (Reference of Registration Committee Meeting in which formulation import was approved).
10. Letter of consent, duly legalized from Indian Embassy/High Commission/ Consulate in the Country of origin **(Applicable in case of 9(4) TI/FI Applications)**
11. List of products for which the registration has been given to the firm and Manufacturing License obtained and products actually manufactured during the previous 2 (two) years **(Applicable in case of 9(4) TI/FI & FIM Applications.)**
12. Relevant Affidavit/Undertakings **(Applicable in case of Bio pesticides Applications)** as under:-
 - (a) Affidavit on bio-pesticide composition on NJSP duly notarized.

- (b) Notarized copy of depositing microbial bio-pesticides strain sample in National Repository with reference code number.
- (c) Undertaking on NJSP duly notarized that product do not contain any genetically modified organism in the prescribed format.
- (d) Undertaking on NJSP duly notarized that product is free from chemical / botanical pesticides / other agro-chemicals.
- (e) Copy of 9(3B) Registration certificate, if relevant .

ANNEXURE - II

**DATA REQUIREMENTS FOR CHEMICAL
PESTICIDES**

Data Requirements for Chemical Pesticides:

A. Chemistry:

Sl No	Parameter	9 (3B)			9 (3)				9 (4)			
		TI	TI M	FIM	TI	TIM	FI	FIM	TI	TIM	FI	FIM
1	2	3	4	5	6	7	8	9	10	11	12	13
A. CHEMISTRY												
1.	Source of Supply of Technical	R	NR	R	R	NR	R	R	R	NR	R	R*
2.	Chemical Composition (clearly showing claims of purity of active ingredient, impurities or adjuvants, as the case may be) in Form-I and L/L	R	R	R	R	R	R	R	R	R	R	R
3.	Physical and Chemical Properties of the active ingredient in Technical and adjuvants in case of a formulation	R	R	R	R	R	R	R	NR	R	NR	NR
4.	Technical Bulletin	R	NR	NR	R	NR	R	NR	NR	NR	NR	NR
5.	Product Specification in	R	R	R	R	R	R	R	NR	R	NR	NR

	BIS format/BIS No. if published											
6.	Method of Analysis	R	R	R	R	R	R	R	NR	R	NR	NR
7.	Analytical Test Report (ATR) from GLP/NABL accredited laboratory	R	R	R	R	R	R	R	NR	R	NR	NR
8.	Characterization (Identity Test) of active ingredient by UV-VIS, IR, MS and NMR spectra)	R	R	NR	R	R	NR	NR	NR	R	NR	NR
9.	Identification & Quantification of Impurities	R	R	NR	R	R	NR	NR	NR	R	NR	NR
10.	Shelf-life claim	R	R	R	R	R	R	R	R	R	R	R
11.	Storage Stability Data (samples stored in three varied agro-climatic conditions) for six months in excess of claimed shelf-life alongwith meteorological data for corresponding period	R	R	R	R	R	R	R	NR	NR	NR	NR

12.	Establishment of Chemical Equivalence	NR	NR	NR	NR	R*/NR*	R*/NR*	R*/NR*	R	R	R	R
13.	Detailed stepwise manufacturing process (provide chemical reactions explained with structural formulae, all applicable reaction other conditions in case of technical grade insecticides including flow sheet diagram).	R	R	R	R	R	R	R	NR	R	NR	NR
14.	Information about Raw Materials Used along with their source of supply	R	R	R	R	R	R	R	NR	R	NR	NR
15.	Effluent Treatment method with complete details	R	R	R	R	R	R	R	NR	R	NR	NR
16.	Legalized letter of consent that the manufacturer is registered for the Technical Grade Pesticide/ Insecticide and	R	NR	R	R	NR	R	R	R	NR	R	R

	that he consents supplying the Technical Grade Pesticide/ Insecticide to the applicant.											
17.	Registration Certificate from DNA of the pesticides from source country	R	NR	NR	R	NR	R	NR	R	NR	R	NR
18.	In case the supplies are to be made through a supplier, a duly legalized certificate from the exporting manufacturer that the supplier is his authorized agent and that the invoice would originate from the approved source of import (actual manufacturer).	R	NR	NR	R	NR	R	NR	R	NR	R	NR
19.	An In-Process sample to be drawn from the R&D/manufacturing facility of applicant in case of technical indigenous	R	R	R	R	R	R	R	NR	R	NR	NR

	manufacture of the insecticide/sample to be submitted in case of technical import ^s ; along with certified reference material (CRM) and standard impurities along with purity certificate in case of technical grade insecticides for pre-registration verification in the Central Insecticides Laboratory, Faridabad (CIL). In case of FIM category sample and CRM to be submitted in CIL Faridabad												
20.	Methodology for residue estimation in BIS format.	R	R	R	R	R	R	R	R	NR	NR	NR	NR

Remarks on chemistry parameters:

1. S. No. (6) & (7) R* wherever If BIS published than not required if not published Registered product specification is required
2. S. No. 12. Not required (NR)- For first Registration, Required (R)-

for subsequent registration after the First Registration.

3. Adjuvant(s) shall be mentioned by their common names(s) and not by code names or numbers and their complete chemical identity shall be provided. No patented adjuvant shall be incorporated in the claimed chemical composition.
4. Sample shall be drawn in case of 9(3) TI, TIM and 9(4) TIM as per approved procedure in 345th RC, a proposal may be sent to DA&FW for their concurrence of drawl of In-Process sample from the technical manufacturing site of importer, till then submission of sample in CIL in case of TI shall be permitted.
5. In case of the insecticides for Seed Treatment, 'Adhesion to Seed Test' shall be invariably provided.
6. Same method of analysis should be used in generating ATR and shelf-life data.
7. Accelerated Storage Data can be considered for grant of provisional shelf-life. However, in such cases the Certificate of Registration (CR) shall be issued with a validity of two years. Shelf-life claim of up to 2-years or as the case may be (provisionally) be granted to the insecticides with a condition that applicant is required to submit real time / actual storage stability study data in the proposed construct and container of sale for duration of minimum 30 months, within two and half years of submission of application for granting the registration, failing which Registration Certificate shall stand invalid.
8. Data requirement for registration of Long Lasting Insecticide Impregnated/ Incorporated Mosquito Bed Nets shall be for 9(3b) and 9(3) category only.
9. Data requirement for registration of Petroleum derived products like spray oil natural mineral oil products shall be for 9(3b) and 9(3) category only.
10. Data at S. No. 7-9 shall not be older than 5 years on the date of application in case of TI.
11. In case of FI-WRT (Formulation Import without registering Technical) OR FIM-WRT (Formulation Indigenous Manufacture without registering Technical), in addition to data on formulation, complete chemistry data on technical including shelf life and packaging data of technical as per the guidelines of TI/TIM should also be submitted along with chemical composition on Rs 10/- Non-Judicial Stamp Paper (NJSP). The applicant of FI-WRT are encouraged to seek registration for Technical also.
12. In case of 9(3b) TI, 9(3) TI/FI & 9(4) TI/FI category, a condition in CR to be incorporated as an analytical test report by the manufacturer (exporting to India) about the quality of the insecticide/Pesticide from a NABL or ISO 17025:2017 compliance /GLP

certified laboratory. (Such analytical test report in respect of the batch(s) shall accompany each & every consignment exported to India)

13. § It was decided that the in-process sample also be drawn in case of TI/FI-WRT. A proposal shall be submitted to DA&FW for in-principle approval. Till such approval is obtained existing policy should be continued.

B. Bio-efficacy: (Insecticide/Fungicide/Herbicide) :

Table-1

Sr. No.	Parameters	9 (3b)			9 (3)					9 (4)
		TI	TI M	FIM	TI	TIM	FI	FI M	Label expansion	TI/TIM/FI/FIM or endorsement of already approved label expansion.
Bio-efficacy										
1.	Bio-effectiveness	R [§]	R [§]	R*	R [§]	R [§]	R**	R**	R**	No data requirement . The claim will be granted as per approved formulation u/s 9(3)/ approval claims of label expansion.
2.	Phytotoxicity	R [§]	R [§]	R*	R [§]	R [§]	R**	R**	R**	
3.	Effect on germination of Seed (in case of seed treatment of fungicide and insecticide)	R [§]	R [§]	R*	R [§]	R [§]	R**	R**	R**	
4.	Effect on parasitoids and predators (in case of Insecticides and PGR)	NR	NR	R*	NR	NR	R**	R**	R**	
5.	Effect on beneficial soil micro-organisms & physico-chemical properties (Herbicide only). Effect on	NR	NR	R*	NR	NR	R**	R**	R**	

	beneficial soil micro-organisms in case of Seed treatment and on soil applied pesticides (Fungicides, Insecticide and PGRs).									
6.	Translocation in Plants	R	R	NR	R	R	NR	NR	NR	
7.	Metabolism in Soil	R	R	NR	R	R	NR	NR	NR	
8.	Metabolism in Water	R	R	NR	R	R	NR	NR	NR	
9.	Metabolism in Plant	R	R	NR	R	R	NR	NR	NR	
10.	Persistence in Soil	R	R	R	R	R	R	R	NR	
11.	Persistence in Water	R	R	R	R	R	R	R	NR	
12.	Persistence in Plant	R	R	R	R	R	R	R	R	
13.	Compatibility with other chemicals, if claimed	NR	NR	R	NR	NR	R	R	R	
14.	Residues in Plant	NR	NR	R #	NR	NR	R#	R#	R	
15.	Residues in Soil	NR	NR	R #	NR	NR	R #	R#	NR	
16.	Residue tolerance limits fixed by foreign countries	NR	NR	R	NR	NR	R	R	R	
17.	Cost Benefit Ratio/ Per Rupee return (per ha)	NR	NR	NR	NR	NR	R	R	R	
18.	Registration status in foreign countries	NR	NR	R	R	NR	R	R	R	NR
19.	MRL Performa along with Pen drive in duplicate (other	NR	NR	R	NR	NR	R	R	R	

	than seed treatment).									
20.	Label and Leaflets (as per Insecticides Rule 18 and 19)	R	R	R	R	R	R	R	R	NR

Remarks on Bioefficacy parameters:

- a. **At S. No. 5-** Applicant may submit position paper and published data related to toxicological effects on beneficial insects in case FI/FIM.
- b. **R*:** Two seasons/years data generated at minimum two different agro-climatic zones
- c. **R**:** Two seasons/years data generated at minimum three different agro-climatic zones
- d. **R#:** One season / year data generated at minimum four different agro-climatic zones for fungicides, insecticides and PGRs. **Whereas, in case of herbicides, two seasons/years data generated at minimum two agro-climatic zones in case of 9(3b) and two seasons/years data generated at minimum three agro-climatic zones in case of 9 (3).** In case of seed treatment one season / year data generated at minimum three different agro-climatic zones will be required. In case of soil applied Pesticides (Insecticide/ Fungicide/ Herbicide), if residue at harvest is above LOQ then 2nd season data will be required or else one season data will be sufficed.

Note: For commercial non edible crops ((like jute, jatropha, rubber, etc.,) only, the data on residue and persistent is plant is not required.

- e. **R\$:** If Technical Import & Technical Indigenous Manufacture application which are submitted together with Formulation Import & Formulation Indigenous Manufacture applications, no bio-efficacy data required.

Note: In case of herbicides data on effect on soil Physico-chemical and biological properties and effect on normally cultivated three succeeding crops is required along with residue studies in the same plots of the field.

Example: for a herbicide intended to be registered for use in wheat crop data on effect on succeeding crops of maize at location one, green gram at location two and sesamum at location three may be generated along with residue studies. However, this

is only an example and data on any other normally cultivated succeeding crop may be generated.

1. For registration of Combination products of two / three registered pesticides (All sort of combination should be addressed in this): data is required as per the guidelines of Formulation Import / Formulation Indigenous Manufacture. However, data on Sr. No. 1, 2 (if any individual component is not registered as formulations, the two seasons comparable data are required to be generated against target insects/ diseases/ weeds using stable / viable formulations. Already registered formulations of individual component of the formulation at approved dosage shall also be included as check), 4, (required only for insecticides and PGR) 10, 11, 12, 13, 14, 15, 16, 17, 18 & 19 (Table - 1) if the a.i. content of either component of the combination product is higher than the already registered same formulations combinations, the applicant is required to submit the data.

Note: S. No. 14 & 15: Data on residue required for as many seasons and locations as required in case of existing FIM u/s 9(3) guidelines. In case of herbicides data on effect on succeeding crops and soil physico-chemical and biological properties are also required along with residue studies as detailed in existing FIM under section 9(3) bioefficacy guidelines for herbicides.

S. No. 10, 11, 12, 14 & 15: These data shall only be required when the concentration / a.i. dose are higher than already registered and / or in case new compound is formed and / or any adjuvants / diluents (other than water) / carrier is different that are in te registered formulations of the individual components. Data on 12, 14, & 15 are also required for the additional crop label claims to common of the individual registered.

2. If technical is already registered for import under section 9(3) and applicant want to apply for indigenous manufacture of the same pesticide then one season data on Sr. No. 1, & 2 (Table - 1) if any, on two representative crops at two different agro-climatic zones is required and one season residue data on two

representative crops particularly on fruits and vegetables is required.

3. If formulation is already registered for import or for indigenous manufacture u/s 9(3) by any applicant and the same registrant want to apply for technical indigenous manufacture, data on Sr. No. 1, 2 & 14 (Table - 1) not required. Whereas, if other applicants want to apply for technical indigenous manufacture, one season data on Sr. No. 1 & 2 (Table - 1) on two representative crops at two different agro-climatic zones is required and one season data on Sr. No. 14 (Table - 1) on two representative crops particularly on fruits and vegetables is required.
4. In case of Technical Import from New Source, two seasons data on each crop mentioned in labels/ leaflets at least at two different agro-climatic Zones is required on Sr. No. 1 & 2 (Table - 1) and two years or seasons data on Sr. No. 14 (Table - 1) on representative crops of each group on which pesticide is approved.

Note: Data on Sr. No. 1 & 2 (Table - 1) is required on all registered formulations of same technical on all approved crops at the time of Issue of import permit provided the application for registration is received within 4 years of issue of import permit.

5. For registration of pesticide formulation for indigenous manufacture having the identical chemical composition that of formulation already registered for import U/s 9(3). No data on bioefficacy is required provided the technical of the source to be used in formulation is duly registered as per guidelines of the Registration Committee and the label claims are same. Whereas, for registration of pesticide formulation for indigenous manufacture having different chemical composition that of formulation already registered for import u/s 9(3), complete data on Bioefficacy to be submitted as per already existing guidelines for formulation indigenous manufacture (FIM) U/s 9(3) provided the technical (source) to be used for making formulation is duly registered as per guidelines of the Registration Committee.
6. In case of Formulation Import without registering technical or Formulation Indigenous Manufacturing without registering technical, in addition to data on formulation, complete bioefficacy data on technical as per the guidelines of

Technical Import or Technical Indigenous Manufacture is required.

7. For Petroleum derived spray oil (PDSO), in case of 9(3) and 9(3b) data on Sr. No. 1, 2, 4, 13, 14, 15, 16 & 18 only as per Table - 1 is required.
8. For Manufacturing Use Product (MUP) of pesticide u/s 9(3) (Other than fruit ripening agent):
 - a. If the technical grade pesticide is not registered, complete data with respect to product technical on Bioefficacy as per guidelines for technical import u/s 9(3) to be submitted along with data on MUP as listed below.
 - b. If the technical grade and source for import is duly registered as per guidelines of the registration committee, no data on Bioefficacy will be required except Registration status of MUP in foreign countries.
 - c. Rationale for import and registering the MUP to be submitted in Bioefficacy.
9. For use of Surfactant with registered pesticide Formulations, data on two seasons Sr. No. 1 & 2 (Table - 1) from minimum three different agro-climatic zones are to be generated with surfactant (tank mix) and without surfactant. The data on Sr. No. 10, 11, 12, 14 & 15 (Table - 1) are to be generated with surfactant as per the requirement of the general guidelines u/s 9(3) of the Insecticides Act, 1968.
10. For registration of twin pack of two registered Herbicides and Surfactant, data on Sr. No. 1, 2 & 13 (Table - 1) if proposed to mix, Sr. No. 10, 11 & 12, 14, 15, 18 & 19 (Table - 1) is required.

Note: Two season data on Sr. No. 1 & 2 (Table - 1) are to be generated on combination of the two herbicides with & without surfactant and individual herbicide from minimum three different agro climatic conditions. The bioefficacy data on other parameters are to be generated on the combination of the two herbicides with surfactant as per the requirement of general guidelines u/s 9(3) of the Insecticide Act, 1968.

11. MRL fixation may be required for seed treatment products on those crops (such as leafy vegetables, etc.) which are being consumed within one month of sowing.

Note: For Fungicides the efficacy trials should be conducted in areas where the claimed crop is the main crop of that particular area and the area should be hot spot of the claimed disease i. e disease pressure should be more than 30 % during the trial period and the fungicide should show minimum 70 % control. This must be certified by the SAU/ICAR institute where the study has been done. In case of insecticide reduction over control should not be less than 70% and in case of herbicides Weed Control Efficiency (WCE) of individual weed species should be minimum 70%, which is duly authenticated by ICAR/SAUs.

Data requirements for Registration for Post-Harvest Treatment (PGR) of crop produce:

a) Data requirements for Registration of Technical -

Note: The data should be submitted on Parameters for Bio-efficacy as per the guidelines approved by the RC from time to time for registration of Chemical pesticides under TIM / TI U/s 9(3) category, as the case may be. The specific data requirements / information for registration of technical (TC/TK) are as under: -

- (i) Fate and behavior in treated crop produce
- (ii) Fate and behavior in air
- (iii) Fate and behavior in water
- (iv) Fate and behavior in soil & data on Sr. No. 16 & 18 (Table - 1) is required.
- (v) If the product technical is not in gas form and/or also to be used to make formulation(s) for use for other than fumigation of post-harvest crop produce (e.g. for use in field crops, household purposes etc.), the additional data requirements on technical to be submitted as per the guidelines approved by the RC from time to time for registration under TIM / TI category U/s 9(3), as the case may be.

b) Data requirements for Registration of MUP (Manufacturing Use Product) (Not for Ripening agent) -

Note: -

- 1) If technical grade of the pesticide is not registered, complete data with respect to product technical on Bio-efficacy as per above guidelines under I. above for technical indigenous manufacture (TIM) / technical Import (TI) U/S 9(3), as the case may be, to be submitted along with data on MUP as listed below.
- 2) If the technical grade and source for import is duly registered as per guidelines of the Registration Committee, data on MUP to be submitted as listed below:
 - (i) Registration status of MUP in other countries.
 - (ii) If the product MUP is not in gas form and/or also to be used to make formulation(s) for use other than fumigation of post-harvest crop produce (e.g. for use in field crops, household purposes etc.) the additional data requirements for registration of MUP to be submitted as per

the guidelines approved by the RC from time to time for registration of Chemical Pesticides U/s 9(3).

c) Data requirements for Registration of formulation -

Note:

- 1) For registration of a formulation under FIM / FI U/s 9(3) category, the technical (TC/TK) and / or MUP from which formulation to be manufactured, should be registered under the Insecticides Act, 1968.
- 2) An applicant seeking registration of a formulation without registering technical for import/indigenous manufacture U/s 9(3), required to submit complete data as per the guidelines for registration of formulation listed below, along with complete data as per above guidelines for registration of technical for import/indigenous manufacture U/s 9(3) category, as the case may be.
- 3) The data parameters for Bio-efficacy shall be as per the guidelines approved by the RC from time to time for registration of Chemical pesticides under FIM / FI U/s 9(3) category, as the case may be. The specific data requirements / information for registration of formulation on post-harvest crop produce (PGR) are as under: -

Table - 2

1.	Data on bio-effectiveness Adverse effect on postharvest crop produce, if any (phytotoxicity, change in appearance and flavour etc.)	For label claim of controlled atmospheric conditions data on 1. And 2. To be submitted for three repeated trials (same temperature and relative humidity) OR For label claim of ambient atmospheric conditions data to be submitted for minimum three repeated trials.
2.	Residue and persistence at different interval (immediately after treatment, start from 0 hours till BDL or acceptable level) in post-harvest crop produce.	For label claim of controlled atmospheric conditions (same temperature and relative humidity) data to be submitted for three repeated trials. OR For label claim of ambient atmospheric conditions (same temperature and relative humidity) data to be submitted for minimum three repeated trials.
3.	Registration status of formulation in other countries	Required
4.	MRL Performa and label leaflets	Required
5.	Label claims as per product Bioefficacy information as per gazette notifications issued by Govt. of India from time to time.	

Note: Post harvest application of 1-MCP should be under expert supervision (PCO's).

Remarks- Appropriate placement and comments from ICAR\NCIPM\SAU

- 1) Bio-efficacy data on target pests (diseases, insects, nematodes and weeds etc.) generated by ICAR/SAUs and institutes under National Agriculture Research System and other institutes approved by registration committee will only be acceptable.

12. Registration of Long Lasting Insecticide Impregnated mosquito bed nets and Long Lasting Insecticide Incorporated mosquito net for registration U/S 9(3) :

1. Three years' bio-efficacy trial in three locations. Out of three locations, two locations should be in endemic areas. The bio-efficacy trial has to be conducted by adopting the protocol devised by the Malaria Research Centre/VCRC (ICMR).
2. Baseline data on persistence of insecticides on the net and its analysis for comparison on yearly basis.
3. Sustainability of fabrics.

13. Registration of insecticides for control of Ecto-parasites (Mites, Bedbugs, Ticks etc.) in poultry u/s 9(3) & 9(3b):

- a) Technical 9(3) & 9(3b) - Data on parameters on Table 1 at Sr. No. 7, 8, 10, 11, 16 & 18 are required.
- b) Formulation 9(3) & 9(3b) - Data on parameters on Table 1 at Sr. no. 13, 16 & 18 are required. In addition to Table 1 at Sr. no. 13, 16 & 18 data on Effect on layers (duration 3 months, study to commence preferably at the age of 40 weeks) - data generated in National/ ICAR/SAU Laboratories [three for 9(3) and 2 for 9(3B)] on [(i) change in body weight, (ii) feed intake, water intake, Feed Conversion Ratio (FCR) (iii) mortality and morbidity pattern, (iv) * Clinical symptoms/morphological changes in organs, (v) * Blood profile and Enzymology, (vi) egg production records for 15 days.], Persistence and residue on treated surface, Residues in various organs of birds and edible products, Residue in birds excreta. (Rearrange)

Note: In case of import of formulation without registering technical, whole set of data on technical shall be submitted along with the application.

14. Registration of insecticidal formulations for use in aircraft disinfection:

1. All insecticides for aircraft disinfection must be manufactured only from the technical grade insecticides which are registered under the Insecticides Act, 1968
2. Data on insecticides formulation shall be considered along with the data on technical grade insecticides and not in isolation.

Bioefficacy and residue data: Bioefficacy test on the proposed formulation should be conducted in Indian conditions minimum 2 trials in each of 3 National Laboratories / NABL accredited laboratories, recognized by Government of India.

Data on persistence of pesticides on commonly used surfaces in the aircrafts and concentration in air, as applicable, should be generated in three National Laboratories/ NABL accredited laboratories.

15. Data requirement for registration of insecticides for use in public health programme u/s 9(3):

1. Bioefficacy data generated by ICMR / MOH&FW Institutes based on multi-centric three years / seasons as per their protocol.

16. Data requirements for registration of house hold pesticides :

- a. All household pesticides as defined must be manufactured only from the technical grade pesticides which are registered under the Insecticides Act, 1968.
- b. Data on household pesticides formulation shall be considered along with the data on technical grade pesticides and not in isolation.

A. Bio-efficacy claims In case of Formulation:

a. Bio-efficacy claims to be given on the labels as under:

- (i) A brief direction concerning the major usages of the pesticides should be given on the labels.
- (ii) Whenever the Registration Committee has approved the product for restricted use, this should be indicated very clearly on the labels in capital letters. 'For use only.
- (iii) Instruction regarding Insecticide 'Not to be used on any food crop to be given'.

b. Bio-efficacy claims to be given on the leaflets:

- (i) Detailed information on the usages of insecticide indicating the name of insects, method of application, dosage, places of treatment, PP equipments to be used etc. should be given in paragraphs forms. Common name of the insects should be given.
- (ii) Whenever the registration committee has approved the product for restricted use, this fact should be indicated very clearly on the leaflets in bold letters.
- (iii) Instructions regarding Insecticides 'Not to be used on any food crop to be given'.

B. Bio-efficacy claims to be given on the labels and leaflets in case of technical grade material: The purpose of import /manufacture of technical grade material is required to be given on the labels and leaflets.

C. Data requirements on Bioefficacy and Residues for formulation of pesticides for provisional registration of U/s 9(3B).

(I) The applicant should submit published / cited Indian data on bio-effectiveness in support of the claims indicated on the labels / leaflets. The data should be produced from 2 National laboratories based on minimum 2 repeated trials. This should be further supported with any published information available from elsewhere (overseas data).

(II) Information on secondary pests outbreaks particularly of ticks and mites should be given where residual pyrethroids are being used.

(III) Data on Residues: Data on persistence, of the pesticides which should be on different types of surfaces should be submitted / generated obtained under foreign / Indian conditions from 2 laboratories. This may also be supported by data generated elsewhere.

(IV) Data on concentration of a.i. in Air - for Aerosols (e.g. Coil, mats, liquid vaporizer etc.). Registration Status in foreign countries.

D. Data requirements on Bio-efficacy & Residues data requirement for regular registration of formulations of pesticides U/s 9(3):

- i. Bio-efficacy test on the proposed formulations should be conducted under Indian **conditions minimum on two trials in each two national laboratories with three replications. In case major difference is reported then data from third National laboratory is required.**
- ii. Data on persistence of pesticides on different types of surfaces should be generated in three national laboratories wherever applicable.
- iii. Information on secondary pests outbreaks particularly of ticks and mites should be given where residual Pyrethroids are being used.

- iv. Data on concentration of a.i. in Air - for Aerosols (e.g. Coil, mats, liquid vaporizer etc.). (Added based on approval in 266th meeting of RC held on 20-07-2006)
- v. Registration Status in foreign countries.

E. Data requirements for the registration of new formulations of the approved pesticides: Data on bio-effectiveness and persistence on different surfaces as required in case of Registration of formulations for regular registration under section 9(3).

F. Data required for combination products: Bio-efficacy data on combination products v/s individual products. Data on persistence on different types of surfaces should also be submitted. All above data should be generated as per the requirement indicated in case of pesticides required for regular registration under section 9(3).

G. Methodology:

Flying/crawling insects:

Residual films of insecticides prepared by spraying insecticides on different types of surfaces, such as Glass, Wood, Mud & Cement surfaces. Insects to be exposed for 30 minutes and then shifted to recovery chambers for 24 hours after which the mortality count should be made and the satisfactory mortality of insects would be more than 90%. The residual toxicity of insecticides should also be studied at different intervals. Evaluation of space spray against flying insects should be conducted in PEET GRADY Chamber as per **standard ISI specification 1824**, mats/coils could also be evaluated inside the Peet Grady Chambers against caged mosquitoes and the knock down effect is to be recorded at different intervals. Aerosols are to be evaluated inside a standard room. The test is to be conducted as per **WHO technical reports series No. 206**.

C. TOXICITY

Sl No	Parameters	Technical		Formulation		Technical/ Formulation	
		9 (3b)	9 (3)	9 (3b)	9 (3)	9 (4)	9 (4)
		TI/TIM	TI/TIM	FIM	FIM/FI	TIM	TI/FIM/FI
1.	Acute oral Rat	R	R	R	R	NR	NR
2.	Acute Dermal-Rat/ rabbit	R	R	R	R	NR	NR
3.	Acute inhalation	R	R	R	R	NR	NR
4.	Primary Skin Irritation	R	R	R	R	NR	NR
5.	Acute Eye Irritation	R	R	R	R	NR	NR
6.	Skin Sensitization Test	R	R	R	R	NR	NR
7.	Repeated dose range finding oral toxicity study (28 days)*	R	R	NR/R	NR/R	NR	NR
8.	Repeated dose 90 days oral (Rat)	R	R	NR/R	NR/R	NR	NR
9.	Repeated dose dermal toxicity	R	R	NR/R	NR/R	NR	NR
10.	Repeated dose inhalation toxicity*#	R	R	NR/R	NR/R	NR	NR
11.	Acute Neurotoxicity-Rodent*	R	R	NR	NR/R	NR	NR
12.	Repeated dose Neurotoxicity-Rodent*	R	R	NR	NR/R	NR	NR
13.	Delayed Neurotoxicity-OP compound-Acute exposure	R	R	NR	NR/R	NR	NR
14.	Delayed	R	R	NR	NR/R	NR	NR

	neurotoxicity- OP compound- Repeated Administration						
15.	Developmental Neurotoxicity*	R	R	NR	NR/R	NR	NR
16.	Developmental toxicity study- a) Rat & b) Rabbit	R	R	NR	NR	NR	NR
17.	Mutagenicity **	R	R	NR	NR	R ⁽⁹⁾	NR
18.	Pharmacokinetics and Metabolism- Rat	NR	R	NR	NR	NR	NR
19.	Pharmacokinetics and Metabolism in other mammals and its similarities or differences from humans.	NR	R	NR	NR	NR	NR
20.	Immunotoxicity study*	NR	R	NR	NR	NR	NR
21.	Acute Avian toxicity *	R (Two species)	R (Two species)	R (One species)	R (One species)	NR	NR
22.	Repeated dose Avian toxicity (One species)	R	R	NR	NR	NR	NR
23.	Avian Reproduction toxicity (One species)	R	R	NR	NR	NR	NR
24.	Acute toxicity to Fresh Water Fish (One species)	R	R	R	R	NR	NR
25.	Acute toxicity- Freshwater invertebrate	R	R	R	R	NR	NR

26.	Acute Toxicity- Honey Bee & (Oral Contact)	R	R	R	R	NR	NR
27.	Effect on Soil microbial activity	R	R	R	R	NR	NR
28.	Acute Toxicity- Earthworm	R	R	R	R	NR	NR
29.	Algal toxicity- Tier I	R	R	R	R	NR	NR
30.	Medical data	R	R	R	R	NR	NR
31.	Human toxicity information from foreign countries	R	R	R	R	NR	NR
32.	Operators/ workers exposure assuming (i) Recommended Personal Protective Equipment (PPE) is Used (ii) Without use of recommende d PPE	NR	NR	NR	R	NR	NR
33.	Health records of Industrial workers	NR	R	NR	R	NR	NR
34.	International report on Carcinogenicit y & Genotoxicity	NR	R/NR	NR	NR	NR	NR

Notes on Toxicity parameters:-

9) Repeated dose 90 days oral (Dog)*- Peer reviewed published literature is acceptable for dog study, same as in case of chemical pesticide data requirement.

Point no.-16, 17, 18)

- i. The Combined Carcinogenicity and Chronic Toxicity study in Rat and
- ii. Separately carcinogenicity study in mice is required.

19)- No separate footnote is required because feeding and metabolism study can be done together

20)- Some metabolites can be more harmful than parent compound even in very small quantity due to bio-activation so it can be conditional requirement.

*The replacement alternatives not involving experiments on animals should also be given due consideration, in case of alternative if available with full and sound justification is provided specifically case to case basis.

*Waiver should be considered when existing information provides robust and scientifically sound weight of evidence approach, which will include examination of all existing data of human & animal toxicity and other information/data (in-chemico, in-vitro, ex-vivo, in-silico models) and read across/bridging from structurally and/or biologically related similar pesticides specifically case to case basis.

- 1) **Technical (TI and TIM) u/s 9(3b) and 9(3)** :- In case of Technical, name and Percentage of relevant and toxic impurities and/or metabolites should be indicated. Also data/information should be provided about their toxicity.)
- 2) **Technical u/s 9(3)** :-
 - a. **-TI u/s 9(3)** :- In case of Technical Import, data from Sl. No. 1 to 40 are required.
 - b. **-TIM u/s 9(3)** :- In case of Technical Indigenous Manufacture, data of Sl. No. 37 and S. No. 39 are not required.
 - c. **Technical Import from new Source u/s 9(3)** :- Data on parameters 1 to 11; 21, 36-37 and 40 is required.
 - d. Technical Indigenous Manufacture u/s 9(3) in case same technical is registered for import or formulation made from the same technical is registered for import or indigenous manufacture:

- I. If impurities are identified , quantified and impurities are within limits (i.e. within maximum of already registered technical) ,then data on parameters 1-6 and 21 is required.
 - II. In case impurities which are not toxic and not relevant and are not within maximum limits of registered technical however are within +3%) and no new impurity is there, then same data as above (Parameters 1 to 6 and 21) will be required.
 - III. If impurities are identified, quantified and are not within limits of registered technical or if impurities are not within +3%) and/or any additional impurity is present then in addition to the tests as indicated in (4) above, additional tests based on the nature and quantity of impurity and QSAR alert will be taken on case to case basis.
- e. **Technical indigenous manufacture u/s 9(3) in case same technical is registered for import or formulation made from the same technical is registered for import BY THE SAME APPLICANT WITH SAME COMPOSITION, PROCESS OF MANUFACTURE ETC.:- Data on Ames Test only is required.**
 - f. **9(4) TIM:-** In case of Technical indigenous Manufacture u/s 9(4), data on Sl. No. 21 data of @ *AMES Test (Tier-I)* is required. (Only AMES test is required)
 - g. **Formulation u/s 9(3):-** For FIM :- as indicated in table except data of Sl. No. 37 and S. No. 39 are not required.
 - h. **In case of FI WRT (Formulation Import without registering Technical) OR FIM WRT (Formulation Indigenous Manufacture without registering Technical):-** in addition to data on formulation, complete toxicity data on technical as per the guidelines of TI , or TIM (as the case may be) should also be submitted.
 - i. **9(4) TI/FIM:-** No data is required, in case of Technical Import(TI) or Formulation Indigenous Manufacture (FIM) u/s 9(4) application.
 - j. **For LLIN:** Acute toxicity studies (Six pack) i.e. S. No. 1 to 6 are required with Premix and health monitoring studies in users as per approved protocol is required with final product (LLIN) .
 - k. **MUP:**
 - a) Data on the parameters from S. No. 1 to 6, should be submitted.
 - b) If the Technical grade pesticide from which MUP is to be prepared is not registered, complete data with respect to product Chemistry (along with sample and reference standard

of technical grade pesticide and impurities), Bioefficacy, Toxicity and Packaging as per the applicable guidelines for registration of technical should be submitted.

1. Household Pesticide Formulations:

- a) Data on the parameters from S.No. 1 to 6, should be submitted for pesticides in Solid & Liquid form. In case of Pesticides in Vapour form or which emits vapour/fumes, in addition to parameters from S.No. 1 to 6, Health monitoring study of the user by using the household pesticides in its actual use should also be submitted. The study should be as per the protocol approved by the RC.
- b) Data on household pesticides formulation shall be considered along with the data on technical grade pesticides and not in isolation.
- m. **Pesticide Formulation for use in Public Health :** Data on the parameters from S. No. 1 to 6, 27, 28, 30 & 31 should be submitted. Recommendations from National Vector Disease Control Program, M/o Health & Farmers Welfare are also required.
- n. **Formulation for use for Aircraft Disinfection:** If the technical grade is duly registered as per guidelines of the Registration Committee, data on the parameters from S.No. 1 to 6 should be submitted.
- o. **Herbicides in twin pack with no other herbicide or with surfactant:** MSDS and Acute toxicity information on surfactant should be submitted along with data on herbicide formulation.

* PLEASE REFER TO GUIDANCE DOCUMENT ON TOXICOLOGY FOR REGISTRATION OF CHEMICAL PESTICIDES IN INDIA.

** In Mutagenicity test; an Ames test, any two *in-vitro* and one *in-vivo* Mutagenicity tests are required.

Repeated Dose Inhalation toxicity Study for 90 days exposure would be required if there is likelihood of significant repeated inhalation exposure as in case of gas, vapours , aerosols, fumigants or likely duration of human exposure via inhalation is long viz. Mosquito coils; sprays used repeatedly.

D. PACKAGING

S1	Parameter	Section 9 (3B)	Section 9(3)	Section 9(4)
.				

		TIM	FIM	TI	TIM	FIM	T I	FI	TIM	FIM	TI	FI
1.	Labels and Leaflets per IR-1971, all fields (as applicable) and as amended from time to time	R	R	R	R	R	R	R	NR	NR	NR	NR
2.	Manner of labeling and Leaflet	R	R	R	R	R	R	R	R	NR	NR	NR
3.	Type of packaging (Ultra small, small or Big whichever is applicable)	R	R	R	R	R	R	R	R	NR	NR	NR
4.	Manner of packaging	R	R	R	R	R	R	R	R	NR	NR	NR
5.	Specification for primary, Secondary and Transport packages (whichever is applicable)	R	R	R	R	R	R	R	R	NR	NR	NR
6.	Details of packaging material and its compatibility with content	R	R	R	R	R	R	R	NR	NR	NR	NR
7.	Performance of container with content during storage stability test (Shelf life Study)	R*	R*	R*	R	R	R	R	NR	NR	NR	NR
8.	Transport worthiness test	R*	R*	R*	R	R	R	R	NR	NR	NR	NR

Chapter V of the Insecticides Rules 1971 in the Insecticides Act, 1968, the rule 16 to 20 of the said chapter deals with the Packaging and Labeling.

R*- Before Commercialization the data will be required.

Note:

1. In case of additional packaging endorsement applications (already approved packaging), the data at Sl. No. 05, 06, 07 & 08, are not required if similar packaging (material) is being sought by the applicant as has been granted to earlier 9(3) registrant.
2. Specification of Bureau of Indian Standard (BIS) must be followed for all the packaging requirements (Wherever available and applicable).
3. All Packaging tests must be carried out with the product of same batch and in its commercial package preferably in Indian condition.
4. The duration of the test and the conditions including geographical conditions must be mentioned.
5. Storage stability data should be generated keeping at least the following parameters in test protocol such as test temperature, test duration, test packaging material, content of active ingredient (a.i.) in the product during and after storage, test humidity, exposure to light, physical and chemical properties of the product during and after storage etc.
6. The testing protocols must have their basis in the WHO/FAO/CIPAC/ASTM recommendations or other validated methodology of GLP/NABL accredited laboratory having packaging testing (chemical / mechanical as applicable etc.) in the scope.
7. The Accelerated Storage Study (ASS) test must be conducted at $54^{\circ}\text{C} \pm 2^{\circ}\text{C}$ (wherever applicable) containing corrosiveness study of the product in reference to packaging material for 14 days as per FAO/WHO manual for claiming appropriate shelf life of the product which can be maximum two years, subject to the condition of providing the ambient storage stability study data of thirty months or as the case may be within thirty months from the date of application for the registration.

ANNEXURE- III

**DATA REQUIREMENTS FOR MICROBIAL
PEST CONTROL AGENTS (MPCA)
PESTICIDES**

Bio-pesticides/ Microbial Pest Control Agents (MPCA)

1. The applicant needs to submit MOU/license agreement between the applicant and the inventor (either own R&D Laboratory or outsourced Research Institute/Facility) or Authorization letter from the inventor of strain OR undertaking by the applicant about the name of inventor/source of strain as per **Annexure-I**.
2. Undertaking declaring that the product is free from Chemical pesticides/Botanicals pesticides/Other Agro-Chemicals as **Annexure-II**.
3. Undertaking on bio-pesticides composition as **Annexure-III**.
4. Updated Stakeholder list for all members in Association/Organization claiming for MOU/authorization for data/technology utilization for mass multiplication/commercialization of the strain, **if applicable**.
5. Form-I dully filled and signed giving complete details along with requisite fee as applicable.
6. Notarized copy of BOD Resolution/ affidavit in case of proprietor/ partnership deed in case of partnership firms.
7. Correct composition as per earlier 9(3) / 9(3B) registrant of bio-pesticide strain.
8. The applicant should also, submit notarized copy of the Permanent Account No. (PAN), allotted by the Income Tax Department.
9. In case of company, the Certificate of Incorporation granted by the Registrar of Companies.
10. Fee receipt issued by NBAIM, Maunath Bhanjan, UP after submission of sample for DNA verification.
11. Undertaking that the product is free from GMO as **Annexure-IV**

The Data Requirements for Microbial Pest Control Agents (MPCA)
Pesticides

- i) Entomopathogenic/ Entomotoxic Bacteria
- ii) Antagonistic Bacteria
- iii) Entomopathogenic Fungi
- iv) Antagonistic Fungi
- v) Nuclear Polyhedrosis Virus (NPV) & Granulosis Virus (GV)

A. Chemistry:

Sl. No.	Characteristics	Microbial (Antagonistic bacteria, Entomopathogenic/Entomotoxic bacteria, Entomopathogenic fungi, Antagonistic fungi, and			
		Primary culture/ mother		Formulated product	
		9 (3B)	9 (3)	9 (3B)	9 (3)
1.	Systematic name (Genus and species)	R	R	R	R
2.	Strain name	R	R	R	R
3.	Common name, if any	R	R	R	R
4.	Source of origin	R	R	R	R
5.	Specification of the product containing Habitat, Physical appearance and morphological description, pH, particle size.	R	R	R	R
6.	Isolation of strain & Manufacturing process	R	R	R	R
7.	Methods of analysis including Quantitative analysis	R*	R*	R*	R*
8.	Shelf life claims	R	R	R	R
9.	Data on storage stability as per shelf life claims	NR	R	NR	R

10.	Composition of the product	R	R	R	R
11.	Potency of product by bioassay method (LC 50 (Beta, Delta , Cry toxin endotoxin content, classification (delta	NR#	NR#	NR#	NR#
12.	CFU/g or ml	R	R	R	R
13.	POB/Capsule count pr ml/g of the product	R%	R%	R%	R%
14.	Adjuvants	NR	NR	R	R
15.	Human pathogens (culture method)	R	R	R	R
16.	Percent content of the Bio-control mass/organism in the formulation & nature of biomass.	R	R	R	R
17.	Percentage of carrier/filler, wetting/dispensing agent, stabilizers/ emulsifiers, contaminants/ impurities	R	R	R	R
18.	Moisture content	R	R	R	R
19.	Contaminants: Pathogenic contaminants such as Salmonella, Shigella, Vibrio and such other microbial, not to exceed 1×10^4 count per ml or per g of formulation	R	R	R	R
20.	Undertaking for free from Chemical and botanical pesticide contaminants	R	R	R	R
21.	Natural occurrence of the organism	R	R	R	R
22.	PCR / Immunology assays ELISA Test	NR [§]	NR [§]	NR [§]	NR [§]
23.	Separation and purification of crystals	NR	NR	NR	NR

24.	A sample for verification (500 ^e g or 500 ^e mL as the case may				
	a. DNA finger printing for the strain verification from NBAIM, Mau Bhanjan.	R	R	R	R
	b. Pre-registration verification at Central Insecticide laboratory (CIL)	R	R	R	R

*Test procedure and criteria used for identification -

- morphology, biochemistry, serology/ Immunology for Entomotoxic bacteria.
- # and \$ these parameters are required for Entomotoxic bacteria.
- % these parameters are required for Virus.
- @ Two samples of same batch of 500 gm/ml each along with copy of the Fee receipt shall be submitted to Central Insecticides Laboratory, Faridabad for PRV purpose by the applicant for Entomotoxic bacteria.
- POB/Capsule count per ml/g of the product only for NPV.
- Viral unit: NPVs 1×10^9 POB/ml or gm. minimum, GVs: 5×10^9 capsules /ml or gm minimum.
- Dual culture to attain at least 50% reduction in target organism (35% for antagonistic bacteria). Bioassay: based on diseased severity and root colonization.
- Natural occurrence of the organism, Immunology assays: Elisa and Separation and purification of crystals are required for Entomotoxic bacteria.
- Test procedure and criteria used for identification by DNA test (Restriction enzymes analysis test).
- Biological assays for determining the LC₅₀ / LD₅₀ of the formulation for Entomopathogenic Viruses. Production of Entomopathogenic Viruses at commercial- scale was done exclusively *in-vivo* by culturing large number of larvae of host insect and subsequently feeding them with semi-synthetic diet contaminated with virus inoculums in laboratory. Viruses production *in-vitro* by culturing insect cells in bioreactors was a substitute for labour intensive maintenance of the massive host-insect colony.
- Manufacturing process including type of fermentation and biological end products. The microbial cultures are multiplied by liquid solid fermentation. Information

pertaining to use of entire mycelia mats with spores separated must be provided in terms of biomass.

Documents to be mandatorily furnished by applicant applying u/s 9 (3)/ 9(3b) for all categories of bio pesticides

1. Verification of the Authorization letter submitted by the applicant via e mail by Secretariat from the original inventor/source of the strain for data utilization for mass multiplication.
2. MOU/license agreement between the applicant and the inventor (either own R&D Laboratory or outsourced Research Institute/Facility) or Authorization letter from the inventor of strain OR undertaking by the applicant about the name of inventor/source of strain as per **Annexure-I**
3. Updated Stakeholder list for all members in Association/Organization claiming for MOU/authorization for data /technology utilization for mass multiplication/commercialization of the strain.
4. Relevant Affidavit/Undertakings: -
 - a) Affidavit on bio-pesticide composition on NJSP duly notarized.
 - b) Notarized copy of depositing microbial bio-pesticides strain sample in National Repository with reference code number.
 - c) Undertaking on NJSP duly notarized that product do not contain any genetically modified organism in the prescribed format.
 - d) Undertaking on NJSP duly notarized that product is free from chemical/botanical pesticides / other agro-chemicals.
 - e) Affidavit of strain Innovator or applicant
 - f) Copy of 9(3B) Registration certificate, if relevant

Note:

1. Bt products should be labelled with bio potency and (or) toxin content. In addition, the labels will have to contain a measurement of toxin protein as percent protein, referring to the Lepidopteran-active toxin(s) present in the crystal.
2. The presently used Bt var. kurstaki standard is HD-1-S-1980

and its potency was calculated at 16,000 IUs per milligram of powder (Beegle et al. 1986. Standardization of HD-1-S-1980: US Standard for Lepidopterous-active *Bacillus thuringiensis*. Bulletin Ent. Soc. America 32: 44-45.). This standard strain is now available with PDBC, Bangalore and DOR, Hyderabad.

3. Defined potency and toxin concentration - Bioassay would require the use of an insect species. Normally manufacturers could select *Trichoplusia ni* / *Helicoverpa armigera* for Lepidopteran specific Bt formulations. *Spodoptera* Units (SPU), *Leptinotarsa* Units (LTUs) or International Toxin Units (ITUs) are to be used for denoting a specific insect.
4. No test for beta exotoxin is required for *Bacillus sphaericus*, because this species is not known to produce exotoxins.
5. The biopotency of products based on *B. thuringiensis* subsp. *israelensis* (*Bti*) is compared against a reference strain IPS82, 1884 using early fourth-instar larvae of *Aedes aegypti* (strain Bora Bora). The toxicity of IPS82 has an arbitrarily assigned toxicity of 15,000 ITU/mg powder.
6. The biopotency of products based on *B. sphaericus* (*Bsh*) is determined against a reference standard SPH88, strain 2362 using early fourth-instar larvae of *Culex pipiens* (strain Montpellier). The toxicity of SPH88 has an arbitrarily assigned toxicity of 1,700 ITU/mg of the powder (Guidelines for laboratory and field testing of mosquito larvicides, WHO 2005 pp 45).
7. The use of alternative bacterial reference powders and / or strains must be approached cautiously. Such alternatives must be the subject of careful cross- calibration against the reference powders and should be conducted by recognized laboratories and should be made available to anyone who wishes to use, or check, the test with the alternative powders/strains.
8. Water content should not exceed 8 %, (12% in *Pseudomonas spp*) to preclude premature degradation of the product.

Guideline for already registered formulation/Strain u/s 9(3) under the IA, 1968.

Applicant shall submit only one folder containing the following documents:

- I. Form-I dully filled and signed giving complete details along with requisite fee as applicable.
- II. Notarized copy of BOD Resolution/ affidavit in case of proprietor/ partnership deed in case of partnership firms.
- III. Correct composition as per earlier 9(3) / 9(3B) registrant of bio-pesticide strain.
- IV. The applicant should also, submit notarized copy of the Permanent Account No. (PAN), allotted by the Income Tax Department.
- V. In case of company, the Certificate of Incorporation

- granted by the Registrar of Companies.
- VI. Authorization letter from the inventor of strain OR undertaking by the applicant about the name of inventor/source of strain as per **Annexure-I**.
 - VII. Requisite number of stamped envelopes.
 - VIII. Copies of Label Leaflets of the product as approved by RC of already registered strain.
 - IX. Copy of letter of Accession No. of strain or information on Accession number of strain.
 - X. Undertaking declaring that the product is free from Chemical pesticides/Botanicals pesticides/Other Agro-Chemicals as **Annexure-II**
 - XI. One sample of 500 gms/ml quantity shall be deposited to NBAIM, Maunath Bhanjan for test relating to DNA fingerprinting particularly partial gene code sequencing of desired strain and a fee may be paid directly to NBAIM through DD or online.
 - XII. Original fee receipt issued by NBAIM, Maunath Bhanjan, UP.
 - XIII. Undertaking that the product is free from GMO as **Annexure-IV**
 - XIV. Undertaking on bio-pesticides composition as **Annexure-III**
 - XV. Toxicology data shall be accepted from Non-GLP laboratory also for encouraging of new strain registration/any new or repeat studies for old strain. This decision shall be applicable to all categories of biopesticide registration, henceforth. The applications under scrutiny in the Secretariat of CIB&RC are also covered under this decision.
 - XVI. A sample of 500 gms/ml shall be deposited in the Secretariat of CIB&RC along with File/documents for PRV purposes.
 - XVII. The above folder shall be scrutinized by the Chemistry division of the Secretariat of CIB&RC.
 - XVIII. No preliminary scrutiny is required for applications for already registered strain of bio-pesticides.
 - XIX. A letter may also be written by the Secretariat of CIB&RC to the Director, NBAIM, Mau Nath Bhanjan, UP for submitting the DNA finger print report directly to the Secretariat of CIB&RC, certifying that the DNA of strain submitted by the applicant (Strain No.) matches with original Strain or otherwise.
 - XX. RC also decided that any government laboratory willing to undertake such studies on the terms and conditions as approved by the committee may request Secretariat of CIB&RC so as to seek approval from RC.

Annexure-I

AFFIDAVIT ON BIO-PESTICIDE STRAIN BY INVENTOR OR APPLICANT

I, S/o, agedyears, resident ofand Proprietor/Authorized person of the firm M/s.....having its office at do hereby declare and solemnly affirms as under:

That I am in the capacity of of firm M/s do hereby declare that the information furnished with respect to composition in Form-I, Label/Leaflet and bonafide verification of the application fir registration of (Name of the product)....., CFU/PBO.....per gm or ml min; Strain No. (Name and number of registered strain)..... invented by M/s.is registered under section 9(3b) or 9(3) of the Insecticides Act, 1968.

1. That a shelf life of the product shall be twelve/Six/four months.
2. The product shall be packed as per IS: 8190 (Part-I) 1988 for Solid Pesticide (Second Revision).
3. That there will be no change in chemical composition, shelf-life, packaging requirement and the product will have the quality and packaging as per the relevant IS or as per specification approved by Registration Committee for 9(3b) & 9(3) registrant.

I, shall be responsible for adhering to the above composition and strain while manufacturing and marketing the product for distribution or sale. In case of any violation of the above declaration and also the conditions laid down on the Certificate of Registration of the said Bio-Pesticide, interalia, Product Quality Speciation submitted by us and also to the specification as and when the same are formulated and published by BIS amendments thereof, I am liable to be prosecuted/rejection of application under the provisions of Insecticide Act, 1968 and the Rules 1971 and amendments thereof.

Deponent

VERIFICATION

I,, the above deponent do hereby verify that what has been declared above is true to the best of my knowledge and belief and nothing has been concealed there from.

Deponent

Annexure-II

**UNDERTAKING FOR ABSENCES OF CHEMICAL/ BOTANICAL PESTICIDES/
CONTAMINANTS/OTHER AGRO-CHEMICALS;**

I, S/o, agedyears, resident ofand Proprietor/Authorised person of the firm M/s, having its office at do hereby declare and solemnly affirms as under:

That the product, (Name of the product)..... formulation containing CFU/PBO/Delta endotoxin content..... per gm or ml min, Strain code, (if any).....Strain No: manufactured by (Name of the applicant)..... does not contain any Chemical/Botanical Pesticide/Contaminants/other Agro-Chemicals.

That I/we shall provide the samples of our product (Name of the product) as and when desired by the competent Authorities of Government of India for verification.

That my/our above undertaking is true, and no portion is false and I have concealed nothing relevant to the above matter.

Place & date: Deponent Signature:

Name Designation: Company Seal:

Annexure-III

AFFIDAVIT ON BIO-PESTICIDE COMPOSITION

I, S/o, agedyears, resident ofand Proprietor/Authorised person of the firm M/s, having its office at do hereby declare and solemnly affirms as under:

That I am in the capacity of..... of firm M/s..... do hereby declare that the information furnished with respect to composition in Form-I, Label/Leaflet and bonafide verification of the application for registration of (Name of the product), CFU/PBO.....per gm or ml min; Strain No. (Name and number of registered strain)..... under section 9(3b) or 9(3) of the Insecticides Act, 1968 is as under:-

1. COMPOSITION: (SPECIMEN FOR Pseudomonas fluorescence WP) Components:

A. Quantity	:	(% w/w)
a) Pseudomonas fluorescence	:	CFU 1x10 ⁸ CFU/gm min 1.0%
b) Carboxy methyl cellulose	:	1.0%
c) Talc Powder	:	98.0%
Total	:	100.0%

2. That a shelf life of the product shall be twelve/Six/four months.

3. The product shall be packed as per IS:8190 (Part-I) 1988 for Solid Pesticide (Second Revision).

4. That there will be no change in chemical composition, shelf-life, packaging requirement and the product will have the quality and packaging as per the relevant IS or as per specification approved by Registration Committee for 9(3b) registrant.

5. Bonafide declare that M/s, manufacturing premises proposed/located at having Registration Certificate total no., if any or Nil and manufacturing license no. if any or Nil.

I shall be responsible for adhering to the above composition while manufacturing and marketing the product for distribution or sale. In case of any violation of the above declaration and also the conditions laid down on the Certificate of Registration of the said Bio-Pesticide, interalia, Product

Quality Speciation submitted by us and also to the specification as and when the same are formulated and published by BIS amendments thereof, I am liable to be prosecuted/rejection of application under the provisions of Insecticide Act, 1968 and the Rules 1971 and amendments thereof.

Deponent

VERIFICATION

I,, the above deponent do hereby verify that what has been declared above is true to the best of my knowledge and belief and nothing has been concealed there from.

Deponent

Annexure-IV

UNDERTAKING BY MANUFACTURERS OF MICROBIAL PESTICIDES

I,-----, aged-----years, s/o-----, R/o-----
-an-----of M/s.-----Registered Office at-----
--do hereby undertake as follows:

That the product-----based on-----Strain No.-----,
manufactured by M/s.-----and /or imported by M/s.....does not
contains any genetically modified organism (GMO) .

- a) That I/We shall abide by the provisions contained in the International Plant Protection Convention with regard to the import of this product.
- b) That I/We shall abide by the provisions in context of International Standards for Phyto-Sanitary Measures- Code of Conduct for the import and release of exotic bio- pesticides of the International Plant Protection Convention (IPPC), FAO, Rome/Plant Quarantine (regulation of Import into India) order,2003.
- c) That I/We shall provide the samples of our product as and when desired by the competent authorities of Government of India for verification.
- d) That I/We further undertake that in the event of the above product having proved otherwise by any competent authority and resulting in environmental damage, I/We shall inform to Plant Protection Adviser, Dte. of

PPQ&S, Sectt. of Central Insecticides Board and
Registration Committee, and other relevant authorities
for Manufacturing Licensing, Pollution Control and of
appropriate District/State/National Level and shall
comply with the directions from them.

- e) That my/our above undertaking is true, and no portion
is false and I have concealed nothing relevant to the
above matter.

Date-----

Place:-----

Signature:-----

Name-----

Designation-----

Seal of the Company-----

Bio-efficacy

Sr. No.	Particulars	Primary culture/mother culture		Formulated product	
		9 (3B)	9 (3)	9 (3B)	9 (3)
1	Field studies: Data on bio-effectiveness and phytotoxicity generated at ICAR, SAUs, CSIR / ICMR institutes. The data should be certified either by the Director or Head of the	NR	NR	R **	R***
2	Laboratory studies: The product should be tested at a laboratory under ICAR/SAU/ CSIR/ICMR. 2.1) LC50 values for each insect species under laboratory conditions should be generated at least at two institutes of ICAR, SAUs, CSIR and ICMR. 2.2) Data on LC50 values for each target insect species should be generated at a laboratory under ICAR/ SAU/ CSIR/ICMR	R	R	R	R
3	Data on non-target organism: One season/one year on effect on product against natural parasites/ predators.	NR	NR	R	R

- R = Required, NR = Not Required
- R** - Two seasons/years data on bio-effectiveness from two agro-climatic Zones
- R *** - Two seasons/years data on bio-effectiveness from minimum three agro climatic Zones.
- 2.1) - Applicable for Entomotoxic Bacteria
- - Applicable for NPV & GV.
- Sr. No. 3 - Required in case of Entomopathogenic fungi, Entomopathogenic Bacteria.

Note: No bio-efficacy data required for already registered strains of Bio-pesticides. Certificate of Registration

will be granted as per approved formulation u/s 9(3)

Toxicity

S. No.	Parameters	Microbial (Antagonistic bacteria, Entomopathogenic/Entomotoxic bacteria, Entomopathogenic fungi, Antagonistic fungi, and Baculovirus)			
		Primary culture/mother		Formulated product	
		9 (3b)	9 (3)	9 (3b)	9 (3)
1.	Single Dose Oral - Rat	R	R	R	R
2.	Single Dose Dermal - Rabbit (Toxicity/Infectivity/Pathogenicity)	R	R	R	R
3.	Acute Inhalation (a)	R	R	R	R
4.	Single Dose Pulmonary - Rat (b) (Toxicity/Infectivity/Pathogenicity)	R	R	R	R
5.	Single Dose Intraperitoneal - Rat (c)	R	R	R	R
6.	Single dose intravenous (d)	R	R	R	R
7.	Primary Skin Irritation - Rabbit	R	R	R	R
8.	Primary Eye Irritation - Rabbit	R	R	R	R
9.	Skin Sensitization - Guinea pig	R	R	R	R
10.	Cell culture (d)	R	R	R	R
11.	Human Safety Records (Effect/Lack of effects)	NR	R	NR	R
12.	Toxicity to bird (1 species) (Toxicity/Infectivity/Pathogenicity)	NR	NR	NR	R (Only 1)
13.	Toxicity to Fresh water Fish (Toxicity/Infectivity/Pathogenicity)	NR	NR	NR	R
14.	Toxicity to Honey bees (e)	NR	NR	NR	R
15.	Toxicity to Silkworm (f)	NR	NR	NR	R
16.	Toxicity to Earthworm (g)	NR	NR	NR	R

Note:

- a. Inhalation toxicity study required for registration of entomopathogenic/entomotoxic bacteria
- b. Pulmonary toxicity study required for registration of antagonistic bacteria, antagonistic fungi, entomopathogenic fungi, baculovirus
- c. Intraperitoneal toxicity study required for registration of antagonistic fungi, entomopathogenic fungi, antagonistic bacteria
- d. Cell culture and Intravenous study required for registration of baculovirus. e and f - required for all except antagonistic fungi

e. required for all except entomopathogenic/entomotoxic bacteria

Note: No data required for already registered strain from the same source with same strain designation and accession number

Note: If genome sequence of conserved region of the microbial strains/microbes used as microbial pest control agent is identical with already registered strain then data is not required from toxicity angle.

Formulations developed from similar already registered mother culture using similar ingredient and process of manufacture then no data is required from toxicity.

- (I) The replacement alternatives not involving experiments on animals should also be given due consideration in case alternative is available with full and sound justification is provided specifically case to case basis.
- (II) Waivers should be considered when existing information provides robust and scientifically sound weight of evidence (WOE) approach, which will include examination of all the existing data of human & animal toxicity and other information/data (in chemico, in vitro, ex vivo, in silico models) and read across/bridging from structurally and/or biologically related similar bio- pesticide specifically case to case basis.
- (III) The waivers accepted for toxicological endpoints for chemical pesticides as per the "2017 guidance documents on toxicology for registration of chemical pesticides in India" will also be applicable for biopesticides (Microbial pesticides, botanicals and semio-chemicals/Pheromones) on a case to case basis.

PACKAGING

Chapter V of the Insecticides Rules 1971 in the Insecticides Act, 1968, the rule 16 to 20 of the said chapter deals with the Packaging and Labelling.

Sl. No.	Parameter	Primary culture/mother		Formulated product	
		9 (3B)	9 (3)	9 (3B)	9 (3)
1.	Labels and Leaflets as per IR-1971, all fields (as applicable) and as amended from time to time	R	R	R	R
2.	Manner of labeling and Leaflet	R	R	R	R

3.	Type of packaging (Ultra small, small or Big whichever is applicable)	R	R	R	R
4.	Manner of packaging	R	R	R	R
5.	Specification for primary, Secondary and Transport packages (whichever is applicable)	R	R	R	R
6.	Details of packaging material and its compatibility with content	R	R	R	R
7.	Performance of container with content during storage stability test(Shelf life Study)	R*	R	R*	R
8.	Transport worthiness test	R*	R	R*	R

R*- Before Commercialization the data will be required.

Note:

1. In case of additional packaging endorsement applications, the data at Sl. No. 05, 06, 07& 08, are not required if similar packaging (material) is being sought by the applicant as has been granted to earlier 9(3) registrant.
2. Specification of Bureau of Indian Standard (BIS) must be followed for all the packaging requirements (Wherever available and applicable).
3. All Packaging tests must be carried out with the product of same batch and in its commercial package preferably in Indian condition.
4. The duration of the test and the conditions including geographical conditions must be mentioned.
5. Storage stability data must be generated keeping at least the following parameters in test protocol viz., temperature, duration, test packaging material, content of active ingredient in the product, test humidity, exposure to light (if applicable), physical and chemical properties of the product during and after storage etc.
6. The testing protocols must have their basis in the WHO/FAO/ CIPAC/ASTM recommendations or other validated methodology of GLP/ NABL accredited laboratory having packaging testing (chemical / mechanical as applicable etc.) in the scope.

7. The storage stability data for microorganisms can vary depending on the type of microbes. For Fungi, maximum storage stability study data will be 12 months at ambient temperature. For Gram negative bacteria like *Pseudomonas fluorescens* or other *Pseudomonas* species the maximum storage stability study data should be 8 months at ambient temperature. For spore forming gram positive bacteria like *Bacillus* species the maximum storage stability study data should be 18 months at ambient temperature.

Note: Additional two months' data for six months self-life claim / three months additional data for one year and six months additional data for 18 months shelf-life claim at two/three different agro climatic locations at ambient temperature along with meteorological data should be submitted.

Besides this, the sub-committee also proposes the Guidelines on Consortium of Bio- pesticides.

Guidelines on Consortium of Bio-pesticides.

Efficiency of biocontrol agents could be increased by the development of mixture of compatible strains of different biocontrol organisms by considering the following norms. While developing a consortia formulation, the following needs to be addressed:

1. Compatible strains combination that differs in pattern of plant/site of colonization.
2. Compatible strains combination is broad spectrum of action against different plant pathogens.
3. Compatible strains combination with different modes of action under similar conditions.
4. Compatible strains combination of genetically diverse group to adapt to different pH, moisture, temperature and relative humidity.

The guidelines of Chemistry, Bio-efficacy, packaging for registration of consortia of Bio-pesticides are similar with the guidelines of Bio-pesticides except the following points.

Guidelines of mother culture/Primary culture of already registered bio-pesticides u/s 9(3) category are not required for registration of consortium Bio-pesticides. Only the guidelines of formulated product (Consortium) will be required. **Ratio of**

each strain in the formulation is required.

1. Following toxicology guidelines for consortia of Bio - pesticides is required.

Note:

- a. -Inhalation toxicity study required for registration of entomopathogenic/entomotoxic bacteria
- b. -Pulmonary toxicity study required for registration of antagonistic bacteria, antagonistic fungi, entomopathogenic fungi, baculovirus
- c. -Intraperitoneal toxicity study required for registration of antagonistic fungi, entomopathogenic fungi, antagonistic bacteria
- d. -Cell culture and Intravenous study required for registration of baculovirus. e and f - required for all except antagonistic fungi
- e. - required for all except entomopathogenic/entomotoxic bacteria

Note:-

- a. If genome sequence of conserved region of the microbial strains/microbes which are used in consortia of Bio-pesticides to be used as microbial pest control agent is identical with already registered strain, then data is not required for mother culture but data is required for combination/consortia from toxicity angle.
- b. If any new formulation of microbes is made by using new ingredients with different processes of manufacture than data is required for the formulation.
- c. If any new combination/consortia /Mixture of microbial strains/microbe developed from already registered microbial strain than data is required only for the mixture and not for mother cultures from toxicity angle.

List of Fungi, Bacteria and Viruses for Consortia

1. Sl. No 591. *Trichoderma* spp .(*T. viride* /*T. asperellum*, *T. harzianum*, *T. virens* etc.)
2. Sl. No 589. *Gliocladium virens* *T. virens*,
3. Sl. No 679. *Ampyliomyces quisqualis*,
4. Sl. No 683. *Coniocytrium minitans*,
5. Sl. No 692. *Chaetomium globosum* and
6. Sl.No 693. *Aspergillus niger* (non-pathogenic/biotype)
7. Sl. No 588. *Bacillus subtilis*,
8. Sl. No 590 *Pseudomonas fluorescens*, *P. protegens*, *P. entomophila*,
9. Sl. No 677, *Streptomyces griseoviridis*,
10. Sl. No 678 *Streptomyces lidicus*,
11. Sl. No 684 *Agrobacterium radiobacter* K84.
12. Sl. No 592. *Beauveria bassiana*,

13. Sl. No 593. *Metarrhizium anisopliae*
14. Sl. No 594. *Nomuraea rileyi*, (New name: *Metarrhizium rileyi*)
15. Sl. No 595. *Verticillium lecanii*, (New name: *Lecanicillium lecanii*)
16. Sl.No 675. *Verticilium chlamydosporium*, (New name: *Pochonia chlamydosporium*)
17. Sl.No.689. *Paecilomyces lilacinus*, (New name: *Purpureocillium lilacinum*)
18. Sl.No.718. *Myrothecium verrucaria-nematicide*
19. Sl. No 326. *Bacillus species* (includes *Bacillus sphaericus* (syn: *Lysinibacillus sphaericus*), *Bacillus thuringiensis* var. *galleriae*, *Bacillus thuringiensis* var. *israelensis*, *Bacillus thuringiensis* var. *kurstaki*, *Bacillus thuringiensis* var. *tenebrionsis*, *Bacillus thuringiensis* var. *sandiego*, *Bacillus thuringiensis* var. *tolworthi* and *Bacillus albus*.)
20. Sl. No 596 Granulosis Viruses (GV)
21. Sl. No 597 Nuclear Polyhedrosis Viruses (NPV) (includes *Spodoptera litura* NPV, *Spodoptera frugiperda* NPV, *Heicoverpa armigera* NPV, *Spodoptera mauritia* NPV, *Mythimna separata* NPV,

1. INDIAN STANDARDS ANTAGONISTIC FUNGI DRAFT SPECIFICATIONS

1. Form and appearance
2. pH
3. Composition
 - 3.1 CFU/g of the product
 - 3.2 Percent content of the Biocontrol organism in the formulation & nature of biomass.
 - 3.3 Percentage of carrier/filler, wetting/ dispending agent,
 - 3.4 stabilizers/ emulsifiers, contaminants/ impurities etc.
 - 3.5 Moisture content
- 4.0 CFU counts: *Trichoderma* 2x10⁶CFU/ml or gm. (Stability at 30°C and 65%RH).

5.0 Contaminants:

5.1 Biological Contaminants:

5.1.1 Pathogenic Contaminants: such as gram negative bacteria *Salmonella*, *Shigella*, *Vibrio* etc.: **absent**

5.1.2 Other contaminants should not exceed 1x10⁴/ml or g

5.2 Chemical/ botanical pesticides contaminants: **absent.**

6. Method of analysis:

- 6.1 CFU counts by serial dilution and examination under regular compound research microscope with bright field optics.
- 6.2 Plating for contaminants on specific media
- 6.3 Antagonistic mycolytic capability on target organism by bioassay on plants (Laboratory test).
- 6.4 Bioassay procedure based on diseased severity and root colonization as detailed in Appendix-I

Appendix-I

Bioassay for plant disease antagonists based on disease severity and root colonization.

The target pathogen to be tested against has to be grown in Sand maize medium. The Sand-maize medium is prepared by adding sand 90g, maize 10g. and water 10ml in a saline or any glass bottle of 300ml capacity and then autoclaved twice. Then 5 mycelial discs of the test pathogen are transferred into the bottle and left for incubation for 15 days. Once the culture has grown well, the sand maize medium is mixed along with the fungal growth and 1g from this preparation is used as the inoculum after adjusting the CFU to $1 \times 10^6/g$ by addition of sand.

The plastic cups (5-6 cm diameter) filled with soil and FYM (3:1) have to be used. In each cup the filling should be done upto $\frac{3}{4}$ th level. The pathogen inoculum is mixed with sand has to be applied upto 2cm depth in the plastic cups.

The bio-efficacy of the bio-agent shall be tested by both seed treatment and soil application. For seed treatment, the recommended dose of the formulation has to be used (5 to 10g.). For soil application, the bio-agent is added at the rate

of 1g of formulation (minimum CFU should be the 2×10^6). The germination percentage, disease intensity and seedling vigour are to be recorded.

Another set of plastic cups filled with sterile soil and sterile FYM has to be used to confirm whether the bio-efficacy was due to the isolate of the bio-agent tested or due to the native isolates of the bio-agent present in the soil.

The keys for grading the efficacy mentioned below shall be used (Srivastava et al., 2002). However, for the registration purpose, the bio-agents that are Highly Efficient, Efficient or Moderately Efficient in the plastic cup test under glass house condition (in the presence of pathogen) can be allowed (i.e.) germination percentage of 70% or above, disease incidence of 30% or less can be considered for registration.

Disease Grading Key

Disease incidence (%)	Description	Rating of bio-efficacy of bioagents
0	Germination >90%, no seed rotting, seedling healthy, root and shoot portions well developed	Highly Efficient (HE)
1-15	Germination 80-90%, infection on main as well as lateral roots, seedlings are well developed	Efficient (E)
16-30	Germination 70-80%, development of roots restricted and growth is less compared to Score 1. Infection occurred on roots. Shoot portions developed but growth retarded compared to Score 1.	Moderately Efficient (ME)

31-45	Germination 60-70%, length of roots and shoots short compared to Score 1. Germination of seeds inhibited. 50% of root area infected. Shoot portions also showed infection	Moderately Inefficient (MI)
46-60	Seed germination 50-60%. Development of roots and shoots greatly retarded. Shoot portions also showed infection.	Efficient (I)
Above 60	Less than 50% germination and seed rotting	Highly Inefficient (HI)

For the root colonization assay, the rhizosphere region of the plants tested above have to be collected and the soil adhering to the root surface has to be removed by gently tapping the roots. The root bits have to be cut into 1 cm bits and randomly 25 bits should be selected for each treatment. They have to be plated on (TSM) and the percentage of root bits colonized has to be recorded. This has to be performed in the sterile soil and non-sterile soil. One control treatment without the Biocontrol agent, being tested, should be kept for both the sterile and non-sterile soil to rule out of the possibility of interference of native micro flora in the bio- efficacy assay.

2. INDIAN STANDARDS ANTAGONISTIC BACTERIA DRAFT SPECIFICATIONS

1. Form and appearance
2. pH
3. Composition
 - 3.1 Percent content of the Biocontrol organism in the formulation & nature of biomass

3.2 CFU/g or ml of the product.

3.3 Percentage of other components: carrier /filler, wetting/ dispersing agent, stabilizers/emulsifiers, contaminants/impurities etc.

3.4 Moisture content.

3.5 CFU counts: Minimum 1×10^8 CFU/ml or gm. (Stability at 30°C and 65%RH).

4. Contaminants:

4.1 Biological Contaminants:

4.1.1. Pathogenic Contaminants: such as gram negative bacteria

Salmonella, Shigella, Vibrio etc.: **absent**

4.1.2 Other contaminants should not exceed 1×10^4 /ml or g

4.2 Chemical/botanical pesticides contaminants: **absent.**

5. Method of analysis:

5.1 CFU counts on specific medium.

5.2 Plating for contaminants on specific media

5.3 Antagonistic capability on target organism by bioassay.

5.4 Bioassay procedure based on diseased severity and root colonization as detailed in Appendix-I

APPENDIX- I

Bioefficacy assay for plant disease antagonists based on disease severity and root colonization:

The pathogen to be tested against has to be grown in sand maize medium. The sand-maize medium is prepared by adding sand 90g, maize 10g and water 10 ml in a saline or any glass bottle of 300ml capacity and then autoclaved twice. Then 5 mycelial discs of the test pathogen are transferred into the bottle and left for incubation for 15 days. Once the culture has grown well, the sand maize medium is mixed along with the fungal growth and 1 g from this preparation is used as the inoculum after adjusting the cfu to 1×10^6 /g by addition of sand.

The plastic cups (5-6 cm diameter) filled with soil and FYM (3:1) have to be used. In each cup the filling should be done upto $\frac{3}{4}$ th level. The pathogen inoculum is mixed with sand has to be applied upto 2 cm depth in the plastic cups.

The bio-efficacy of the bio-agent can be tested by both seed treatment and soil application. For seed treatment, the recommended dose of the formulation has to be used (5 to 10g). For soil application, the bio-agent is added at the rate of 1g of formulation (minimum cfu should be the 2×10^6 , the CIB recommended dose). The germination percentage, disease intensity and seedling vigour are to be recorded.

Another set of plastic cups filled with sterile soil and sterile FYM has to be used to confirm whether the bio-efficacy was due to the isolate of the bio-agent tested or due to the native isolates of the bio-agent present in the soil.

The keys for grading the efficiency mentioned below can be used here (Srivastava et al., 2002). However, for the registration purpose, the bio-agents that are Highly Efficient, Efficient or Moderately Efficient in the plastic cup test under glass house condition (in the presence of pathogen) can be allowed (i.e.) germination percentage of 70% or above, disease incidence of 30% or less can be considered for registration.

Disease Grading Key

Disease incidence (%)	Description	Rating of bio-efficacy of bio-agents
0	Germination >90%, no seed rotting, seedling healthy, root and shoot portions well developed	Highly Efficient (HE)
1-15	Germination 80-90%, infection on main as well as lateral roots, seedlings are well developed	Efficient (E)
16-30	Germination 70-80%, development of roots restricted and growth is less compared to Score I. Infection occurred on roots. Shoot portions developed but growth retarded compared to Score I.	Moderately Efficient (ME)
31-45	Germination 60-70%, length of roots and shoots short compared to Score I. Germination of seeds inhibited. 50% of root area	Moderately Inefficient (MI)

	infected. Shoot	
	portions also showed infection	
46-60	Seed germination 50-60%. Development of roots and shoots greatly retarded. Shoot portions also showed infection.	Inefficient (I)
Above 60	Less than 50% germination and seed rotting	Highly Inefficient (HI)

For the root colonization assay, the rhizosphere region of the plants tested above have to be collected and the soil adhering to the root surface has to be removed by gently tapping the roots. The root bits have to be cut into 1 cm bits and randomly 25 bits should be selected for each treatment. They have to be plated on TSM and the percentage of root bits colonized has to be recorded. This has to be performed in the sterile soil and not sterile soil. One control treatment without the biocontrol agent being tested should be kept for both the sterile and non-sterile soil to rule out the possibility of interference of native microflora in the bio-efficacy assay.

For the bacterial antagonists, the above bioassay procedure has to be followed where only the % root colonization will be considered and other parameters are not required. The % root colonization required is 80%.

3. INDIAN STANDARDS ENTOMOPATHOGENIC FUNGI DRAFT SPECIFICATIONS

1. Form and appearance
2. pH
3. Composition
 - 3.1 CFU/g of the product
 - 3.2 Percent content of the Biocontrol organism in the formulation & nature of biomass.
 - 3.3 Percentage of carrier/filler, wetting/ dispersing agent, stabilizers/ emulsifiers, contaminants/ impurities etc.
 - 3.4 Moisture content
4. CFU counts: Minimum 1×10^8 CFU/ml or gm. (Stability at 30°C and 65%RH).
5. Contaminants:
 - 5.1 Biological Contaminants:

5.1.1 Pathogenic Contaminants: such as gram negative bacteria *Salmonella*, *Shigella*, *Vibrio* etc: **absent**

5.1.2 Other contaminants should not exceed 1×10^4 /ml or g

5.2 Chemical/botanical pesticides contaminants: **absent.**

6. Method of analysis:

6.1 CFU counts by serial dilution and examination under regular compound research microscope with bright field optics.

6.2 Plating for contaminants on specific media

6.3 Entomopathogenic capability on target insects by bioassay.

Appendix-I

Laboratory bioassay procedures for screening fungal pathogens on *Spodoptera litura* and *Helicoverpa armigera*

Insect pathogens:

Beauveria bassiana*, *Metarhizium anisopliae*, *Nomuraea rileyi

Preparation of Fungal inoculum for bioassays:

The fungus is grown on SDAY/SMAY medium for 10 days in slants and aqueous spore suspensions of various concentrations are prepared using sterile water. The spore count is estimated by Haemocytometer. (10^4 - 10^{10} spores/ml). Tween-80 is added @ 0.01% to get uniform spore suspension.

Rearing insects:

H. armigera*, *S. litura- Artificial diet

(Semi-synthetic diet) Stage of insect

for bioassay

H. armigera*, *S. litura - II instar larvae to be used for bioassay protocols for lepidopteron pests

Method of inoculation

S. litura

1. Cut castor leaf discs of 3.0cm diameter, rinse in sterile distilled water and place each leaf disc in a sterile Petri plate and allow it air dry in a laminar flow system

2. Apply ten micro liters of the spore suspension of each concentration on the leaf disc and spread it uniformly on the

leaf surface and allow it air dry in a laminar flow system. Treat the other side of the disc similarly.

3. Release ten numbers of second instar larvae of *S. litura* on the leaf surface and incubate the discs in an incubator at 25°C and 90% RH

4. After 24 hours, shift the larvae to the polypots containing the semi-synthetic diet and incubate in an incubator at 25°C and 90% RH

5. After 5 days of incubation, mortality of the larvae are recorded in each concentration tested

6. Lc-50 can be calculated using SPSS package

Standard for LC₅₀: Not more than 2.00X10⁶ spores/ml (3.0X10³ spores/mm²)

***H. amigera*:**

Instead of castor leaves, soybean leaves can be used for ***H. amigera*** and the procedure is same as above.

Standard for LC₅₀: Not more than 4.00X10⁶ spores/ml (6.0X10³ spores/mm²)

Appendix-II

Bioassay procedure for *Plutella xylostella*

Various concentrations of *Beauveria bassiana* formulation ranging from 6 x 10⁸ to 2 x 10¹⁰ are to be screened to assess the mortality.

Fresh undamaged radish leaves free from pesticide application are to be collected and washed thoroughly in sterile distilled water and air-dried. Individual leaves are dipped in respective concentrations for 30 seconds. After complete drying of leaves ten late 2nd instar larvae of *Plutella xylostella* are released per treatment. A water dipped radish leaf is maintained simultaneously as control.

To prevent desiccation of leaves, the petiole is covered with a moist cotton swab. Each treated leaves are placed in a plastic container of dimension 12.5 x 10 cm containing moist filter

paper, Whatman No.41 to provide humidity.

Each treatment has to be replicated thrice. Fresh radish leaves were provided as feed at 24 hours interval. This set up has to be maintained at 25±1°C and 70-80% RH for 7 days. Observations on larval mortality are to be made at 3, 5 and 7 days after treatment.

Standard for LC₅₀ = Not more than 3 x 10⁹ cfu/g

4. INDIAN STANDARDS, ENTOMOTOXIC BACTERIA TECHNICAL /FORMULATION DRAFT SPECIFICATIONS

S.No.	Details
1. SCOPE	
1.1	This Indian Standard prescribes the requirements and the method of sampling and test for Entomotoxic bacteria technical and formulation. The product is a biopesticide active against target insects. The product is not for human consumption.
2. REQUIREMENTS	
2.1	Common name: i.e., <i>Bacillus thuringiensis</i> or <i>B. sphaericus</i> etc.
2.2	Systematic name (Genus, species, serotype, strain and Cry-toxin* along with cry gene)
2.3	Physical specification 2.3.1 Form and appearance 2.3.2 Moisture content 2.3.3 pH
2.4	Composition 2.4.1 Delta endotoxin content (Minimum 2.0%) - estimation as per Appendix-V 2.4.2 Adjuvants 2.4.3 Beta Exotoxin content - Negative through housefly bioassay test as per Appendix-IV 2.4.4 Human pathogens (gram negative bacateria Salmonella, shigella & vibrio etc) - Absent 2.4.5 Other microorganisms (not more than 10 ⁴ / g) 2.4.6 Chemical/botanical pesticide contamination - Absent
2.5	Natural occurrence of the organism 2.5.1 Its relationship of the organisms 2.5.2 History (exotic or indigenous strain)

	2.5.3 The isolate should not be genetically modified organism (GMO).
3. SAMPLING	
3.1	Representative samples of the material shall be drawn in accordance with IS 10946:1984
4. TESTS	

4.1	<p>An appropriate test procedure and criteria used for identification, such as morphology, biochemistry and / or serology / immunology</p> <p>4.1.1 Morphology description, particle size</p> <p>4.1.2 Immunology assays: ELISA / Dot blot assay test or any other sensitive standard immunology test.</p> <p>4.1.3 Method of analysis</p> <p>4.1.4 Level of beta exotoxins to be identified if expressed by Housefly bioassay method.</p> <p>4.1.5 Potency of product by bioassay method (Appendix-II)</p> <p style="text-align: right;">4.1.5.1 Bioassay method</p> <p style="padding-left: 40px;">a) LC 50 on target larvae and potency against a reference using artificial diet or leaf disc method or in the water for mosquito larvae (Appendix-I)</p> <p style="padding-left: 40px;">b) Housefly Bioassay method for Beta-exotoxin content (Appendix-IV)</p> <p style="padding-left: 40px;">c) Determination of toxin content by ELISA / Dot Blot Assay Method (Appendix-V)</p> <p>4.1.5.2 A technique for the separation and purification of the crystals (Appendix III) is to be used by the manufacturer and the antisera to be raised using solubilized toxin. Toxin content (3.5 %) to be standardized in the formulation using this antisera (ELISA /Dot blot assay).</p>
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2.2 Crytoxin* If H-Serotype is not known, it is mandatory to provide the details of Cry toxin to confirm that

it is *Bacillus thuringiensis*.

Appendix I

Bioassay

Method Diet

incorporation

The following protocol is used for diet incorporation of oral toxicants to test their toxicity on target insects. The example presented here is to bioassay Cry I Ac on *H. Armigera* (First instar larva of other test insects are used for similar bioassay).

1. Pipette out 3 ml of the solution into a 40 ml plastic cup.
2. Pour lukewarm diet, approx. 60⁰ C, into the cup to a total volume of 30 ml. Place the lid and shake the cup vigorously for a minute to mix properly.
3. Pour the diet to 0.5 cm height, into wells of a 24-cell insect-rearing tray. Allow the diet to cool in laminar airflow under UV lamps for 1 h to surface sterilize the diet.
4. If concentration of the toxicant in the stock solution was 2 µg/ml, the final concentration in the diet would now be 0.2µg/ml diet. Thus the final concentration of toxin in diet was diluted 10-fold.
5. Release first instars into the diet rearing trays at the rate of one per well. Cover the diet tray with semi-permeable wrap and close the lid.
6. It is recommended that the lid be tightly secured to the tray with rubber bands, to prevent the larvae from escaping. Because the diet is unsuitable, larvae try constantly to escape from the diet rearing trays.
7. Keep controls with larvae released on untreated diet, for all the experiments.
8. The unused rearing trays with diet can be stored in a refrigerator for a week.
9. Change the diet for the larvae every two or three days.
10. Record mortality observations at 8 hourly intervals until the end of seven days, for median lethal time LT₅₀ calculations. LT₅₀ is the time at which 50 % of' the test

population is killed with the specific dose tested. A simple linear regression equation can be worked out to calculate the LT_{50}

11. Otherwise, record mortality at alternate days until the end of seven days, for median lethal concentration LC_{50} calculations. LC_{50} is the concentration that kills half the test population.
12. Record weights of surviving larvae at the end of seven days, for median effective concentration EC_{50} and LC_{50} is the concentration that prevents half the test population from reaching 50% of the weight attained by control larvae. For example, if the average weight of larvae on the control diet (without toxin) was 80 mg, EC_{50} represents the concentration at which 50% of the test population is unable to gain a weight more than 40 mg. LC_{50} is the concentration that inhibits half the test population from reaching the third instar.

Diet incorporation for filter paper bioassays

1. For bioassays with bollworms, 10 ml toxin incorporated diet is poured over a 16 sq cm piece of filter paper. The filter papers layered with diet are cooled and cut into smaller squares of 2 x 2 cm, and 10 first instar larvae are released in small plastic cups 3 x 3 cm (d x h) cups containing a square. Change the strips every alternate day.
2. Record mortality observations until the seventh day.

Surface coating of semi-synthetic diet

1. Prepare the diet and pour it into the trays or the rearing plastic cups. Generally, 10 μ l of the toxin can be used to coat 1 sq cm surface area. Gently swirl the diet surface to ensure uniform and complete spread of the solution over the diet surface.
2. Allow the surface to dry in a laminar airflow under UV light for 2-3 hours to surface sterilize.
3. Release one first instar *H. armigera* larva per well. Always maintain proper controls with untreated diet.
4. Change the diet on alternate days and record mortality until the seventh day. Then, weight of surviving larvae should be recorded on the final day of the bioassay.

The method has the advantage of obtaining constantly reliable results because the toxin is unlikely to be affected by either improper mixing or heat as can occur in the diet-incorporation method. Moreover, less amount of the toxin is required for the assay, compared to the diet- incorporation method.

Calculation of results:

The potency of the sample (International Units - IUs)

LC50 Standard

IU/mg sample = ----- X IU/mg Standard

LC50 Sample

(IU/mg Standard, i.e., HD-1-S-1980 is 16,000 IUs/mg; the US standard is available with PDEC, Bangalore; each registrant should prepare a "self reference" and should deposit it with the Registering Authority. Each self reference will be expressed as IU/mg using International standard)

Exotoxin determination by PCR studies

Methodology:

Some *Bacillus thuringiensis* strains secrete type I β - exotoxin, which is a non-specific insecticidal and thermostable adenine nucleoside oligosaccharide. Toxicity bioassays and HPLC are traditional methods for detecting β -exotoxin. For rapid approach for prediction of type I β -exotoxin production, PCR-based method can be followed as per Diego H Sauko et al. (2014). One of these ORFs encodes the Exo protein that was proved to be responsible for the phosphorylation of a β -exotoxin precursor at the last step of their biosynthesis process (Liu et al., 2010). Primers BEF (forward; 50- CGGCAGCCGTTTATTCAAA-30) and BER (reverse; 50- CCCCTTCCCATGGAGAAACA-30) amplify a 406-bp DNA fragment of thuE between nucleotides 373 and 778. All *B. thuringiensis* strains are grown on nutrient agar plates for 16h. A loopful of cells is

transferred to 100 µl H₂O and boiled for 10 min to make DNA accessible for PCR amplification. The lysate is centrifuged briefly (5 s at 20,000g), and 5 µl supernatant is used as a DNA template in each polymerase chain reaction. This is performed with a final volume of 25 µl containing 2.5 µl 10x reaction buffer, 0.5 µl 50 mM MgCl₂, 0.5 µl 100 mM deoxynucleoside triphosphate mixture, 8 pmol each primer, and 1 U of Taq polymerase (Invitrogen). The PCR amplification consisted of DNA denaturation at 94°C for 2 min followed by 25 cycles of amplification with a gradient thermocycler. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at 54°C for 1 min, and a chain elongation step at 72°C for 1 min. The final elongation step was extended for an additional 5 min. Subsequently, 10 µl PCR product is analysed by 1.0% agarose gel electrophoresis. A positive control can be also used for better results.

Appendix-II

Dot Blot assay of *Bacillus thuringiensis* (B.t.) toxin protein as alternate of Bioassay.

- 1) B.t. grown till sporulation in shake flask or in fermenter vessel and let the cells lyse and release spore/crystals into the medium
- 2) Cells are harvested by centrifugation at 10k for 15 mins.
- 3) Wash the pellet with 1M NaCl to remove the B.t. associated seine/metallo proteases and washd twice with sterile distilled water.
- 4) Pellet suspended in 50MM NaOH to solublize the toxin protein for 2 hours at R.T. with slow shaking and centrifuged again at 10K for 15 Mins.
- 5) Supernatant was adjusted to pH 8.0 with Tris HCL pH 8.8

- 6) Protein contents estimated by Lowry's protocol.
- 7) Two fold serial dilutions of test protein were made in PBS and known amount of protein applied on NCP using S&S or Biorad Dot Blot manifold apparatus and applying water vacuum for 30 mins.
- 8) NCP was carefully removed from Dot Blot set and soaked in excess of 3% Skim milk in PBS for blocking the remaining acetic sites on NCP for 2-3 hours at R.T/O/N at 4°C.
- 9) Wash the NCP with excess PBS with 0.01% Tween 20, 3-4 times and then finally with PBS
- 10) Polyclonal antiserum raised against total crystal protein was suitably diluted in PBS and added to the 'seal a meal' containing NCP and incubated for 1-2 hours with shaking.
- 11) Remove the NCP from the bag and wash several times (as mentioned in step.No.9)
- 12) Anti-rabbit antibodies conjugated with HRPO/alkaline Phosphate was diluted as per the suppliers instruction and incubated NCP (as in step 10)
- 13) Was as in step 11
- 14) For HRPO:
 - a) Diaminobenzene (4mg/10ml PBS)/4-Chloro-1-Naphthol (4mg/10ml 20% Alcohol) were dissolved and 10ml of 30% of H₂O₂ per 10 ul substrate solution was added and colour reaction developed in dark for 5-10 mins (DAB gives brick red colour. 4ON gives blue colour).
 - b) For alkaline Phosphatase:

Alkaline Phosphatase Buffer:

1M Tris pH 8.8	- 10ml/
4M NaCl	- 2.5ml/ make up to 100ml
1M MgCl ₂	- 0.5ml/

For 10ml of above buffer add NBT-66 ul and BCIP-33 ul and developed and colour reaction

15. Stop the reaction by removing the substrate and washing with PBS.
16. Keep on filter paper and dry.

DIFFERENT PROTEIN CONCENTRATION

10ug 5ug 2.5ug 1.25ug 512.5ng 256.25ng 128ng 64ng 32ng
16ng 8ng 4ng

Determination of cell dry weight

- # Take a known volume of Bacterial culture spin down at 4R for min.
- # Wash the pellet in minimal distilled water
- # Transfer to a pre weighed container
- # Incubate at 80°C for 16-18 hours till become dry and weight becomes constant.

Appendix-III

PURIFICATION OF CRYSTALS BY GELATIN METHOD

Centrifuge the sporulated material and wash pellet twice with 1M NaCl. Add 200ml. of 0.5% Gelatin, stir and remove all froth completely. Dilute with sterile water and centrifuge. Take debris and stir with 20ml. of 1.5M sucrose. Further add 50 ml of 1.5M sucrose, stir and centrifuge at 3000 RPM for 2 hours. Remove supernatant and purified crystals are harvested.

2. INDIAN STANDARDS BACULORIVUS DRAFT SPECIFICATIONS

1. Form and composition of the product
 - 1.1 Viral Unit: POB/Capsule count per ml/g of the product
 - 1.2 Percent content of the bio-control organism in the formulation and nature of biomass

- 1.3 Percent of carrier/filler, wetting/dispersing agent, stabilizers/ emulsifiers, containments/ impurities etc.
2. Moisture content
3. pH
4. Viral Unit:

NPVs (*Helicoverpa* & *Spodoptera*) - 1×10^9 POB/ml or gm (minimum)
(POB -Polyhedral Occlusion Body)

GV (*Chilo*, *Plutella* & *Acheae*) - 5×10^9 Capsules/ml or g.
(minimum).

5. Contaminants:

5.1 Biological contaminants:

5.1.1 Pathogenic contaminants: Pathogenic contaminants such as gram negative bacteria *Salmonella*, *Shigella*, *Vibrio* etc. should be **absent**:

5.1.2 Other microbial contaminants: Other microbial contaminants should not exceed 1×10^4 /ml or g

5.2 Chemical/botanical pesticides contaminants should be absent.

6. Identification of Baculovirus by DNA test (Restriction enzyme analysis test).

7. An undertaking should be submitted that the strain is indigenous, naturally occurring and not exotic and not genetically modified as per Annexure-1.1

8. Method of analysis:

Viral Unit:

NPVs (*Helicoverpa* and *Spodoptera*) = 1×10^9 POB/ml or gm. minimum

GVs = 5×10^9 Capsules/ml or gm. minimum.

8.1 In case of NPVs/, POB/Capsule count should be taken with Haemocytometer using shallow depth counting chamber as detailed in Appendix - I

8.2 Biological assay for determining the LC_{50} or LD_{50} of the formulation:

8.2.1 Bioassay for NPV by the Diet Surface Contamination Method as detailed in Appendix-II OR

8.2.2 Bioassay for GV against *Chilo infuscatellus* as detailed in Appendix-III OR

8.2.3 Bioassay for GV against *Plutella xylostella* as detailed in Appendix-IV.

8.2.4 Bioassay for GV against *Acheae janta* as detailed in Appendix-V.

8.3 Plating for contaminants on specified media.

Appendix-I

COUNTING OF NPV/GV (POB/CAPSULE) USING IMPROVED NEUBAUER HAEMOCYTOMETER COUNTING CHAMBER.

A haemocytometer is used for estimating of NPVs/GVs in a unit volume of the product. The Improved Neubauer Haemocytometer comprised a thick glass slide with a shallow depression in the central section divided into two halves (figure-1). Each side, the base of the depression has a fine ruled grid of squares (figure-2) which is visible under a microscope. The dimensions of this grid are defined. Place a standard cover slip placed over the depression and a one half halves of the slide chamber using a micro pipette. The particles require 2-5 minutes to sediment to the chamber floor.

Either dark field or a phase contrast microscope is used to identify and count polyhedral occlusion bodies (POB) or capsule. With the counting chamber under the microscope, the number of Polyhedra/capsule in a given number of grid squares can be counted. Each count consists of a tally of the number of polyhedra completely contained within a big square plus the number of touching the top and left sides. Polyhedra touching the bottom and right sides are not counted. Since both the depth of the chamber and the grid dimensions are known. It is then a straight forward calculation to determine the number of polyhedra /capsule per ml of test suspension.

$$\text{Number of NPV (POB) per ml/gm} = \frac{D \times X}{N \times K}$$

Where:

D = Dilution factor

X = Total number of polyhedra

counted N = Number of squares

counted

K = Volume above one small square in $\text{cm}^3 = (2.5 \times 10^{-7} \text{cm}^3)$

Area of each small square is $1/400 \text{ mm}^2 = 0.0025 \text{ mm}^2$. Depth of chamber is 0.1mm. Volume of liquid above a single small square is $0.0025 \text{ mm}^2 \times 0.1 \text{ mm} = 0.00025 \text{ mm}^3$. To convert to cm^3 multiply by 1/1000 to get a volume of $2.5 \times 10^{-7} \text{ cm}^3$ above 1 small square. Hence, $K = 2.5 \times 10^{-7} \text{ cm}^3$

Worked example:

Suppose in a sample diluted by a factor of 1000 we count 535 polyhedra in 160 small squares then:

D = 1000

X = 535

N = 160

K = $2.5 \times 10^{-7} \text{ cm}^3$

$$1000 \times 535$$

Thus, POB count = $\frac{1000 \times 535}{160 \times 2.5 \times 10^{-7}}$ = 1.34×10^{10} POB/ml of test sample

$$160 \times 2.5 \times 10^{-7}$$

Note: (i) Usually, this procedure is repeated 3 times and an average taken to get a more accurate estimate.

(ii) Same procedure will be used for GV also for counting the number of capsule per unit volume of the product.

PROCEDURE FOR ESTIMATION OF LC₅₀ OF NPV BY THE DIET SURFACE CONTAMINATION METHOD.

- i) **Diet to be used:** The standard chickpea-based diet without formaline.
- ii) **Bioassay bottles:** 5ml. vials with a diameter of 18 mm (255 mm² surface area)
- iii) **Doses of NPV to be tested:**

<i>Helicoverpa armigera</i>		<i>Spodoptera litura</i>	
POB/ml	POB/mm ²	POB/ml	POB/mm ²
-----	-----	-----	-----
a) 5x10 ⁴	1.96	1x10 ⁶	39.21
b) 1x10 ⁴	0.39	2x10 ⁵	7.84
c) 2x10 ³	0.078	4x10 ⁴	1.57
d) 4x10 ²	0.016	8x10 ³	0.31
e) 0.8x10 ²	0.003	16x10 ²	0.062
f) 1.6x10	0.0006	3.2x10 ²	0.013

- iv) **Method of dosing:** Dispense 10 Microlitre aliquots into each vial and spread uniformly over the entire diet surface using a polished rounded lip of 4 mm glass rod and allow to dry off under flow laminar hood for 10 minutes.
- v) **No. of larvae/dose:** 50 (Maintain 50 healthy larvae without virus inoculation for control)
- vi) **Stages of larvae:** II instar larvae (Preferably 4 days old) Release one larva/vial and plug mouth with sterile absorbent cotton. Incubate at 25 ± 1°C for 7 days.
- vii) Record mortality in different doses on the 7th day.
- viii) Apply Abott's formula for correction of mortality in

- control treatment.
- ix) Subject the dose - mortality response to probit analysis using relevant statistical software.
- x) Express LC₅₀ as POB/mm² of diet surface.
-

Expected standards for NPV for II instar larvae

<u>Species</u>	<u>LC₅₀ POB//mm²</u>
1. <i>Helicoverpa armigera</i>	< 0.5
2. <i>Spodoptera litura</i>	< 20.0

Appendix-III

Bioassay for GV against *Chilo infuscatellus*

Determination of LD₅₀:

To determine the LD₅₀ of the GVs, third instar larvae should be used. The larvae are to be microfed (one micro litre per larva) with six different doses, viz. 1.1×10^1 , 10^2 , 10^3 , 10^4 , 10^5 , and 10^6 IBs/larva. One hundred freshly moulted larvae have to be used for each treatment. Larvae fed with equal quantity of distilled water serve as control. The mortality has to be recorded daily. The LD₅₀ of the virus is determined following the probit analysis method (Finney, 1962).

LD₅₀ = < 1×10^3 OB for third instar larvae by micro-feeding.

Appendix-IV

Laboratory bioassay procedures for estimation of LC₅₀ of *Plutella xylostella* (PxGV) by leaf disc method:

1. Cut leaf discs of cauliflower (3.2cm). Soak it in 0.1N NaOCl for 5 min. and wash thoroughly in distilled water. Air dry these leaf discs for 2-3 minutes. (Fifth leaf from top to be used)
2. PxGV (containing 0.01 per cent Triton X 100) of different concentrations 28000, 2800, 280, 28, 2.8 OB/mm² on the leaf disc) is prepared
3. Aliquots of 12ul of each concentration of GV is dispensed on the upper surface of the leaf disc and spread uniformly with a blunt end glass rod (use separate tips and glass rods for each treatment)
4. Air dry these leaf discs for 2-3 minutes
5. Repeat the same on the lower surface of the leaf disc.
6. Control discs were treated with distilled water containing 0.01 per cent Triton X 100 only.
7. The leaf discs are placed in Petri dishes lined with wet filter paper discs and 35-second instar larvae of *P. xylostella* (starved for 6 hours) are released on each leaf disc starting from control treatment to highest concentration. This is replicated three times.
8. Incubate these larvae at 25°C
9. After 24 hours remove the treated leaves (partially eaten) and provide the larvae with fresh cauliflower leaves.
10. The leaves are changed daily and mortality data recorded every day.
11. The dosage and time mortality responses are subjected to probit analysis.
12. If the mortality in the control excess 10% repeat the

experiment.

LC₅₀ = < 0.15 OB/mm² for second instar larvae by disc method.

Appendix-V

Laboratory bioassay procedures for estimation of LC₅₀ of *Achaea janata* Granulosis virus (AjGV) by leaf disc method:

1. Cut leaf discs of castor (8cm dia) and wash in distilled water. Air dry these leaf discs for 5 minutes.
2. Treat the leaf disc on both the upper and lower surfaces with 200 µl suspension of AjGV (containing 0.02% Tween-80) of different concentrations (5x10⁸, 5x10⁷, 5x10⁶, 5x10⁵, 5x10⁴ corresponding to 19884, 1988, 198, 19, 1.9 OB per mm² on the leaf disc)
3. Aliquots of 100 µl of each concentration of GV is first dispensed on the upper surface of the leaf disc and spread uniformly with a blunt end of glass rod (use separate tips and glass rods for each treatment)
4. Air dry these leaf discs for 5 minutes
5. Repeat the same on the lower surface of leaf disc
6. Control leaf discs were treated with distilled water containing 0.02% Tween-80 only
7. The leaf discs are placed in Petri dishes (9.0cm dia) lined on wet filter paper discs and 35 second instar larvae (third day after hatching) of *A. janata* are released on each leaf disc starting from control treatment to highest concentration. This is replicated three times.
8. Incubate these larvae at 25°C.
9. After 24-48 hours remove the treated leaves (partially

- eaten) and provide the larvae with fresh castor leaves
10. The leaves are change daily and mortality data recorded every day
 11. The dosage and time mortality responses are subjected to probit analysis
 12. If the mortality in the control exceeds 10% repeat the experiment.

Recommended LC_{50} GV (*Achaea janata*) - $LC_{50} < 4$ OB/mm² for second instar larvae by the leaf disc method.

ANNEXURE IV

The Data Requirements for Botanical/ Plant origin Pesticides .

II: Plant Based Products

Botanical Pesticides/Pesticides of Plan Origin

Eucalyptol, Cymbopogon formulation, Neem Based Pesticides, Pyrethrum Extract & Rotenone for Pisci-culture (Formulation)] & Essential Oils (formulations only) etc.

General Guidance

1. It is clarified that traditional remedies or products prepared by the individuals for their own-self use do not need registration if the produce is not sold. If the product prepared or the treated produce is to be sold in the market, then the registration is required under the provisions of the Act.
2. Sometimes the active substances to be used as Plant origin or botanical bio-pesticides, are vastly studied and the information of good quality which is consistent with current methodology, information/data is available, then waivers for submission of certain data, if

justified, can be granted. Further, in some cases, based on the information and characteristics or the results of studies, additional data may be required.

3. For registration of extract u/s 9(4): It shall be obtained from the same part of the plant with same process of extraction and should have identical chemical composition as of 9(3) registrant.
4. Formulation to be registered u/s 9(4) should have identical chemical composition and required to be prepared from the extract already registered under the Act for use in the country.

Chemistry

Plant extract like Eucalyptol, Cymbopogon, Neem Based Pesticides, Concentrate Pyrethrum Extract, Herbal/ Botanical plant growth regulator, Rotenone for Pisciculture and Plant essential oil etc.

S. No	Parameter	Botanical					
		Plant extract/ concentrate			Formulation		
		9(3b)	9(3)	9(4)	9(3b)	9(3)	9(4)
1	Name of the Part of the Plant(s) to be used for extraction of the active ingredients.	R	R	R	R	R	R
2	Source of supply of the technical grade material	R	R	R	R	R	R
3	Outline of Process of Formulation.	R	R	R	R	R	R

4	Extract Contents active ingredient	NR	NR	R	R	R	R
5	Chemical composition of the formulations	R	R	R	R	R	R
6	Physico-chemical properties of a.i. & adjuvants	R/ NR	R/ NR	NR	R/ NR	R/ NR	R/ NR
7	Product Specifications as BIS format	R	R	R	R	R	R
8	Method of analysis (a.i.)	R	R	R	R	R	R
9	An undertaking that the product does not contain any other chemical pesticide except plant extract	R	R	R	R	R	R
10	Analytical test report	R	R	R	R	R	R
11	Shelf-life claim	R	R	R	R	R	R
12	Shelf-life data	NR	R	NR	NR	R	NR

Bio-efficacy

	Parameters	Technical			Formulations		
		9 (3B)	9 (3)	9 (4)	9 (3B)	9 (3)	9 (4)
1	Bio-effectiveness against target pest species in specified crops	R* (Two season data from min. Two agro climatic zones)	R* (Two season data from min. Three agro climatic zones)	No bio-efficacy data required. Certificate of	R (Two season data from min. Two agro climatic zones)	R (Two season data from min. Three agro climatic zones)	No bio-efficacy data required. Claim will be
2	Phytotoxicity (as per	R* (same	R* (same as	Registrati	R (same as	R (same	granted as

	standard tests)	as above 1)	above 1)	on will be granted as per approved technical u/s 9(3)	above 1)	as above 1)	per approved formulation u/s 9(3).
3	Compatibility with other agro-chemicals, if claimed	R*	R*		R	R	
4	Stability of formulation (Photo, Thermal etc.) in aqueous dilution (acidic, neutral & basic)	R*	R*		R	R	
5	Direction concerning dosage for each target pest species	R*	R*		R	R	
6	Stage of crop for use and stage of target pest	R*	R*		R	R	
7	Waiting period	R*	R*		R	R	
8	Application equipment	R*	R*		R	R	
9	Information regarding registration status in other countries, if any.	R	R		R	R	
10	Time of Application	--	--		R	R	
11	Purpose of Manufacture	--	--		R	R	
13	Residue in Plant	--	--		R	R	
14	Residue in Soli	--	--		R	R	
15	Registration status in foreign	--	--		R	R	

	countries						
1 6	Residue tolerance limits fixed by foreign countries)	--	--		R	R	

R - Required NR - Not Required.

Note :

1. Sr. No. 4 & 6 not required in case of Cymbopogon formulation (Plant extract) & Neem Based Pesticides
2. Sr. No. 1,2,3,,5,6,7,8,9,10 applicable for Essential oil for Formulation only.

Toxicology

	Parameters	Extract/concentrate			Formulation		
		9 (3b)	9 (3)	9 (4)	9 (3b)	9 (3)	9 (4)
1.	Acute oral toxicity - Rat	R	R	No data required if chemical equivalence is established with 9(3) registrant.	R	R	No data required if chemical equivalence is established with 9(3) registrant.
2.	Acute dermal toxicity - Rabbit	R	R		R	R	
3.	Acute Inhalation - rat	R	R		R	R	
4.	Primary Skin Irritation - Rabbit	R	R		R	R	
5.	Primary Eye Irritation - Rabbit	R	R		R	R	
6.	Skin Sensitization - Guinea pig	R	R		R	R	
7.	Sub-acute oral - rat	NR	NR/R		NR	NR	
8.	Sub - acute oral - dog	NR	R		NR	NR	

9.	Sub-acute dermal	NR	R		NR	NR	
10.	Sub-acute inhalation	NR	R		NR	NR	
11.	Neuro-behavioral toxicity	NR	R		NR	NR	
12.	Teratogenicity	NR	R		NR	NR	
13.	Effect on Reproduction	NR	R		NR	NR	
14.	Carcinogenicity/chronic toxicity/combined toxicity study) *	NR	R		NR	NR	
15.	Metabolism	NR	R		NR	NR	
16.	Mutagenicity* (AMES + 2 in-vitro +1 in-vivo)	NR	R		NR	NR	
17.	Toxicity to birds (2 species)	R	R		R	R	
18.	Toxicity to fresh water fish	R	R		R	R	
19.	Toxicity to Honey bees	R	R		R	R	
20.	Toxicity to Earthworm	R	R		R	R	

21.	Medical data	R	R		R	R	
22.	Human toxicity information	NR/R	NR/R		NR/R	NR/R	
23.	Health record of industrial workers	NR	R		NR	R	
24.	International report on carcinogenicity & genotoxicity study	NR/R	NR/R		NR/R	NR/R	

Note:

1. The requirement for sub-acute studies shall be determined on the basis of results of other toxicity study reports.
2. *PLEASE REFER TO GUIDANCE DOCUMENT ON TOXICOLOGY FOR REGISTRATION OF CHEMICAL PESTICIDES IN INDIA.

PACKAGING

Chapter V of the Insecticides Rules 1971 in the Insecticides Act, 1968, the rule 16 to 20 of the said chapter deals with the Packaging and Labeling.

Sl No.	Parameter	Extract/concentrate			Formulation		
		9(3B)	9(3)	9(4)	9(3B)	9(3)	9(4)
1.	Labels and Leaflets per IR-1971, all fields (as	R	R	R	R	R	R

	applicable) and as amended from time to time						
2.	Manner of labeling and Leaflet	R	R	R	R	R	R
3.	Type of packaging (Ultra small, small or Big whichever is applicable)	R	R	R	R	R	R
4.	Manner of packaging	R	R	R	R	R	R
5.	Specification for primary, Secondary and Transport packages (whichever is applicable)	R	R	R	R	R	R
6.	Details of packaging material and its compatibility with content	R	R	R	R	R	R
7.	Performance of container with content during storage stability test (Shelf life Study)	R*	R	R	R*	R	R
8.	Transport worthiness test	R*	R	R	R*	R	R

R*- Before Commercialization the data will be required.

Note:

1. In case of additional packaging endorsement applications, the data at Sl. No. 05, 06, 07& 08, are not required if similar packaging (material) is being sought by the applicant as has been granted to earlier 9(3) registrant.
2. Specification of Bureau of Indian Standard (BIS) must be followed for all the packaging requirements (Wherever available and applicable).
3. All Packaging tests must be carried out with the product of same batch and in its commercial package preferably in Indian condition.

4. The duration of the test and the conditions including geographical conditions must be mentioned.
5. Storage stability data should be generated keeping at least the following parameters in test protocol such as test temperature, test duration, test packaging material, content of active ingredient (a.i.) and relevant impurities in the product during and after storage, test humidity, exposure to light, physical and chemical properties of the product during and after storage etc.
6. The testing protocols must have their basis in the WHO/FAO/CIPAC/ASTM recommendations or other validated methodology of GLP/NABL accredited laboratory having packaging testing (chemical / mechanical as applicable etc.) in the scope.
7. The Accelerated storage study (ASS) test must be conducted at 54± 2°C (wherever applicable) for 14 days as per FAO/ WHO manual for claiming appropriate shelf life of the product which can be maximum two years, subject to the condition of providing the ambient storage stability study data of thirty months or as the case may be within thirty months from the date of application for the registration.

ANNEXURE V

THE DATA REQUIREMENTS FOR PHEROMONES/SEMIO-CHEMICALS

The Data Requirements for Pheromones/Semio-chemicals Chemistry

Technical for import/indigenous manufacture:

S No	Parameters	9 (3b)	9 (3)	9 (4)
1.	Active ingredient/Tech conc.	R	R	R
2.	Chemical composition	R	R	R
3.	Chemical name (s)	R	R	R
4.	Common name	R	R	R
5.	Physical chemical properties	R	R	R
6.	Manufacturing process	R	R	R

7.	Analytical Test Report from GLP/NABL accredited laboratory	R	R	R
8.	Storage condition with special reference to temperature	R	R	R
9.	Source of import (In case of import only)	R	R	R
10	Sample for PRV purpose	R	R	R
11	Purpose of import/indigenous manufacture	R	R	R

1. TECHNICAL FOR IMPORT/INDIGENOUS MANUFACTURE:

1. Active ingredient
2. Laboratory test: Lure manufactured from the particular ingredient will be tested by using wind tunnel and should demonstrate minimum 50% attractancy.

LURE/DISPENSER FOR IMPORT/MANUFACTURE:

1. Laboratory test; The lure/dispenser should demonstrate at least 50% attractacy using the wind tunnel.
2. Field test: The data on bio-efficacy based on two seasons field trials from two different agro-climatic conditions in the form of authentic/published report.
3. Compatibility: No data on compatibility are required unless the product is recommended for use in combination with pesticides or other agrochemicals.
4. Time and method of application: Information on timing, disruption is to be furnished.
5. Intended uses.
6. Mode of action and degree of specificity.
7. Target pest (s) and crops or premises to be protected.

8. Application rate.
9. Manner, rate and frequency of application.
10. Limitations of use.

Note: As per decision of 248th meeting of R.C. Pheromones used for monitoring and mass trapping are not covered under the various provisions of the Insecticides Act, 1968.

Bio-efficacy

S. No.	Particulars	9 (3b)	9 (3)	9 (4)
1.	Active ingredients	R	R	No bio-efficacy data required. Claim will be granted as per approved formulation u/s 9(3).
2.	Laboratory test (attractancy) : Lure manufactured from the particular ingredient will be tested by using wind tunnel and should demonstrate minimum 50% attractancy	R	R	
LURE/DISPENSER FOR IMPORT/MANUFACTURE:				
3.	Laboratory test: The lure/dispenser should demonstrate at least	R	R	

	50% attractacy using the wind tunnel.		
4.	Field test	The data on bio-efficacy based on two seasons field trials from one agro-climatic conditions in the form of authentic/published report.	The data on bio-efficacy based on two seasons field trials from two different agro-climatic conditions in the form of authentic/published report.
5.	Compatibility: No data on compatibility are required unless the product is recommended for use in combination with pesticides or other agrochemicals.	R	R
6.	Time and method of application: Information on timing, disruption is to be furnished.	R	R
7.	Intended uses.	R	R
8.	Mode of action and degree of specificity.	R	R
9.	Target pest (s) and crops or premises to be protected.	R	R
10	Application rate.	R	R
11	Manner, rate and frequency of application.	R	R
12	Limitations of use.	R	R

Note: As per decision of 248th meeting of R.C. Pheromones used for monitoring and mass trapping are not covered under the various provisions of the Insecticides Act, 1968.

SPECIFICATIONS FOR PHEROMONE TRAPS AND LURES

The committee after detailed discussions on the existing specification of various models of pheromones traps decided not to make any change in these specifications. However, these specifications may further be examined by SAU and SDAS of various States:

1. **Funnel shaped trap:** This trap is generally used for trapping the moths of *Helicoverpa armigera*, *Spodoptera litura*, *Earisa* spp. etc.

Colour: Any colour other than black.

Structure: The funnel trap may have three parts (1) canopy (2) funnel shaped "trap base" and (3) a collection device.

Canopy: Dia : 120-160mm

Thickness : 1.0-3.0mm

(There should be a provision for fixing the canopy to the "trap base" and also the (pheromone lure)

Trap base:

Dia of the mouth : 75-120mm

Height of funnel : 45-190mm

Dia of the bottom hole : 20-30 mm

Should possess a "L" or "T" shaped handle or any other device by which the other device by which the "trap" may be fixed to the support.

The "Trap base" may be provided with 2 to 4 stalks for fixing the canopy to the "trap base". The canopy should be firmly rest on stalks so that the canopy is not dialodged due to wind.

Collection device: It should be made of polythene or other suitable material. It should withstand wind, temperature and rain water.

Should be fixed to the "trap base" in such a way that the device remains attached to the trap under field conditions.

II. **Sticky trap (for pink boll worm etc.):**

- * Corrugated DVC, Plastic laminated card board, tin or any other suitable material that should be water-proof.
- * The sticky glue should be non-drying.
- * The outer surface of trap should be water proof.
- * The colour may be except black.
- * There should be provision for fixing the trap for support.

III. **Fly trap (For fruit/vegetable flies):**

- * Material construction as described in sticky/funnel trap.
- * Any colour except black.
- * Should withstand rainfall, heat/temperature and wind.
- * Should be structured in such a way that the trap is escape proof.

Specification of Lures:

1. Lure made of sulphur free rubber/polypropylene/PVC, Impregnated with specific pheromone blends.
2. Field efficacy should be minimum for 15 days after application.
3. Impregnated lures should be packed singly in individual trilaminated pouches with 30 Ml Aluminum foil.
4. Shelf-life of Lure in original pack should be minimum 6 months at room temperature.
5. Lures should attract insect species only, with 50% insect attractancy by pheromone/lure/dispenser by using wind tunnel method.

Toxicology

S. No	Parameters	Semiochemicals (pheromones)		
		9 (3b)	9 (3)	9 (4)
1.	Acute oral toxicity-rat	R	R	No data required if chemical equivalence is established with 9(3) registrant
2.	Acute dermal toxicity-rat	R	R	
3.	Acute Inhalation - rat	R	R	
4.	Primary Skin Irritation - Rabbit	R	R	
5.	Primary Eye Irritation - Rabbit	R	R	
6.	Skin Sensitization - Guinea pig	R	R	
7.	Sub- acute oral- rat	NR	R	
8.	Sub-acute dermal	NR	R	
9.	Sub- acute inhalation	NR	R	
10.	Neurotoxicity	NR	R	
11.	Teratogenicity	R	R	
12.	Effect on Reproduction	NR	R	
13.	Carcinogenicity/chronic toxicity/combined toxicity study*	NR	R	
14.	Metabolism	NR	R	
15.	Mutagenicity (AMES + 2 in-vitro +1 in-vivo)*	R	R	
16.	Cellular immune response	R	R	
17.	Toxicity to bird (2 species)	R	R	
18.	Toxicity to fresh water fish	R	R	

19.	Toxicity to Honey bees	NR	R	
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Toxicology

Note: 1. Lepidopterean Pheromones that are naturally occurring compound designated by an unbranched aliphatic chain (between 9 and 18 carbons) ending in an alcohol, aldehyde or acetate functional group and containing upto 3 double bonds in aliphatic backbones can be exempted from the sub-acute toxicity carcinogenicity, effect on reproduction and metabolism if their use rates do not exceed 150 gm/acre/year with Good Agricultural Practices (GAP) and used in solid matrix dispensers.

2. For formulation products no toxicity data are required unless it is added with some other pesticides.

3. * **PLEASE REFER TO GUIDANCE DOCUMENT ON TOXICOLOGY FOR REGISTRATION OF CHEMICAL PESTICIDES IN INDIA.**

PACKAGING

Chapter V of the Insecticides Rules 1971 in the Insecticides Act, 1968, the rule 16 to 20 of the said chapter deals with the Packaging and Labeling.

Sl. No.	Parameter	Semiochemicals (pheromones)		
		9(3 b)	9(3)	9(4)
1.	Labels and Leaflets per IR-1971, all fields (as applicable) and as amended from time to time	R	R	R
2.	Manner of labeling and Leaflet	R	R	R
3.	Type of packaging (Ultra small, small or Big whichever is applicable)	R	R	R
4.	Manner of packaging	R	R	R

5.	Specification for primary, Secondary and Transport packages (whichever is applicable)	R	R	R
6.	Details of packaging material and its compatibility with content	R	R	R
7.	Performance of container with content during storage stability test(Shelf life Study)	R*	R	R
8.	Transport worthiness test	R*	R	R

R*- Before Commercialization the data will be required.

Note:

1. In case of additional packaging endorsement applications, the data at Sl. No. 05, 06, 07& 08, are not required if similar packaging (material) is being sought by the applicant as has been granted to earlier 9(3) registrant.
2. Specification of Bureau of Indian Standard (BIS) must be followed for all the packaging requirements (Wherever available and applicable).
3. All Packaging tests must be carried out with the product of same batch and in its commercial package preferably in Indian condition.
4. The duration of the test and the conditions including geographical conditions must be mentioned.
5. Storage stability data should be generated keeping at least the following parameters in test protocol such as test temperature, test duration, test packaging material, content of active ingredient (a.i.) and relevant impurities in the product during and after storage, test humidity, exposure to light, physical and chemical properties of the product during and after storage etc.

6. The testing protocols must have their basis in the WHO/FAO/ CIPAC/ASTM recommendations or other validated methodology of GLP/ NABL accredited laboratory having packaging testing (chemical / mechanical as applicable etc.) in the scope.
7. The Accelerated storage study (ASS) test must be conducted at 54± 2°C (wherever applicable) for 14 days as per FAO/ WHO manual for claiming appropriate shelf life of the product which can be maximum two years, subject to the condition of providing the ambient storage stability study data of thirty months or as the case may be within thirty months from the date of application for the registration.

The abbreviations used in the guidelines are in APPENDIX- I

1. ASS: Accelerated Storage Stability
2. ASTM: American Society for Testing and Materials
3. ATR: Analytical Test Report
4. CFU: Colony Forming Unit
5. CIL: Central Insecticide Laboratory
6. CIPAC: Collaborative International Pesticides Analytical Council
7. CRM: Certified Reference Material
8. CR: Certificate of Registration
9. CSIR: Central Scientific Industry and Research
10. DNA: Designated National Authority
11. ELISA: Enzyme Linked Immunosorbate Assay
12. EPN: Entomopathogenic Nematode
13. FAO: Food and Agriculture Organization
14. FI: Formulation Import
15. FIM: Formulation Indigenous Manufacturing
16. FI-WRT: Formulation Import without registering Technical
17. FIM-WRT: Formulation Indigenous Manufacturing without registering Technical
18. GAP: Good Agriculture Practices
19. GLP: Good Laboratory Practices
20. GV: Granulosis Virus
21. ICAR: Indian Council of Agricultural Research
22. ICMR: Indian Council of Medical Research
23. IR: Insecticides Rules
24. ISI: Indian Standards Institutions
25. LC: Lethal Concentration
26. LD: Lethal Dose
27. LLIN: Long Lasting Impregnated Nets

28. MIC: Minimum Inhibitory Concentration
29. MOH&FW: Ministry of Health and Family Welfare
30. MOU: Memorandum of Understanding
31. MRL: Minimum Residue Limit
32. MUP: Manufacture Use Products
33. NABL: National Accreditation Board of Testing and Calibration Laboratories
34. NBAIR: National Bureau of Agriculture Insect Resources
35. NIBSM: National Institute of Biotic Stress Management
36. NPV: Nuclear Polyhedrosis Virus
37. NR: Not Required
38. PGR: Plant Growth Regulators
39. POB: Polyhedral Occlusion Bodies
40. PVC: Poly Vinyl Chloride
41. R: Required
42. RC: Registration Committee
43. SAU: State Agriculture University
44. SDAS: State Designated Agencies
45. TC/TK: Technical Grade
46. TI: Technical Import
47. TIM: Technical Indigenous Manufacturing
48. VCRC: Vector Control Research Centre
49. WHO: World Health Organization

The terms used in the report are defined in APPENDIX- II

1. **Active ingredient:** The part of the product that provides the pesticidal action.
2. **Applicant:** The party (manufacturer, importer or their representative) that makes an application for registration of a pesticide to the responsible authority. (Please refer to Form-1 foot note under the Insecticide Rule 1971)
3. **Bio-pesticides :** Biopesticides is a generic term generally applied to a substance derived from nature, such as a microorganic or botanical or semiochemical that may be formulated and applied to control the pest and diseases.

- 4. Contaminant or impurity in MPCA:** Any microorganism or substances it produces that are present in a product, other than the specified microorganism (or substances it produces) of the microbial pest control agent (MPCA); an alternate/mutant form of the MPCA is considered to be a microorganism impurity.
- 5. Equivalence :** The determination of the identical similarity of the purity, impurity and toxicological profile as well as of the physical and chemical properties, presented by supposedly similar technical material originating from different manufacturers/place.
- 6. Formulation:** The combination of various ingredients in order to render the product useful and effective for the purpose claimed in the manner recommended.
- 7. Good Laboratory Practice (GLP):** A quality system concerned with the organizational process and the conditions under which non-clinical health and environmental safety studies are planned, performed, monitored, recorded, archived and reported.
- 8. Herbicide,** an agent, usually chemical, for killing or inhibiting the growth of unwanted plants, such as residential or agricultural weeds and invasive species.
- 9. Household pesticides** are commonly used indoors to control pests such as ants, cockroaches, houseflies, mosquitoes, fleas, ticks, bedbugs, termites, rodents, mites and microbes. ... Household pesticides may contain one or a combination of active ingredients of synthetic (chemical) or natural (plant or microorganism) origin.

10. "Import" means bringing into any place within the territories to which this Act extends from a place outside those territories;

11. Infective or Infectivity: The ability of a microorganism to invade and persist in a viable state and to multiply within or on an organism, with or without disease manifestation. The nature of an infection can vary widely with respect to severity, location and number of organisms involved

12. "Insecticide" (Pesticide) means - -

- i)** Any substance specified in the Schedule; or
- ii)** Such other substances (including fungicides and weedicides) as the Central Government may, after consultation with the Board, by notification in the Official Gazette, include in the Schedule from time to time; or
- iii)** Any preparation containing any one or more of such substances.

13. "Label" means any written, printed or graphic matter on the immediate package and on every other covering in which the package is placed or packed and includes any written, printed or graphic matter accompanying the insecticide.

14. "Manufacture" in relation to any insecticide, include :-

- i)** Any process or part of a process for making, altering, finishing, packing labeling, breaking up or otherwise treating or adopting any insecticide with a view to its sale, distribution or use but does not include the packing or breaking up of any insecticide in the ordinary course of retail business; and
- ii)** Any process by which a preparation containing an insecticide is formulated.

- 15. "Package"** means a box, bottle, casket, tin, barrel, case, receptacle, sack, bag, wrapper or other thing in which an insecticide is placed or packed.
- 16. Pathogenicity:** The ability of a microorganism to cause disease and/or inflict damage on the host. Many pathogens cause disease by a combination of (i) toxicity and invasiveness or (ii) toxicity and colonizing ability. However, some invasive pathogens cause diseases that result from an abnormal reaction of the host's defense system.
- 17. Pesticide product:** The formulated product (pesticide active ingredient(s) and co-formulants) in the form in which it is packaged and sold.
- 18. Plant growth regulators** (PGRs) are chemicals used to modify plant growth such as increasing branching, suppressing shoot growth, increasing return bloom, removing excess fruit, or altering fruit maturity.
- 19. Plant extract/concentrate:** a botanical substance produced from the defined source(s) and by the described manufacturing processes, and which is the "active substance". For botanical active substances, the extract will be in most cases a mixture of components from the plant and in addition all components that result from the cultivation, harvest, post-harvest storage and primary processing and manufacturing. It may be difficult to identify and characterize all individual components. Some of these components may be considered as components of concern which may be considered in the same way as "relevant impurities" in chemical pesticide.
- 20. Post-harvest** :In agriculture, postharvest handling is the stage of crop production immediately following harvest, including cooling, cleaning, sorting and packing etc.

- 21. Public Health pesticides:** Pesticides that are used in the control of pests of public health significance under public health programs in the country.
- 22. Registration Dossier:** The set of data that is submitted by applicants, in a structured manner, in support of their application for registration. as per the requirements of the registration committee.
- 23. Semiochemicals :** Chemicals emitted by a plant or animal that evoke a behavioral or physiological response in another organism. When the semiochemical affects an individual of the same species, it is called a 'pheromone'. When it affects an individual of a different species, it is called 'allelochemical'.
- 24. Technical material :** Technical-grade materials and technical concentrates; also known as technical-grade active ingredient (TGAI).
- 25. Technical grade of MPCA:** Microbial material used for manufacture of microbial pest control products. It is the purest preparation of the MPCA resulting from a typical production process, and contains no additives except for purposes of MPCA growth or replication, or typical purification and preparation. It may be commercially distributed to manufacturers of microbial pest control products either in its pure form or augmented with preservatives, stabilizers, and diluents; or it may be a hypothetical stage in the manufacture of the microbial pest control product.