Annexure 10.32.1

Modalities and Guidelines for formulation under import / indigenous manufacturing with registration of technical (first time registration of a molecule).

The proposed requirements in the guidelines as mentioned below and the proposed modalities of its implementations are as under:

1. The guidelines once approved shall be applicable for the insecticide/pesticides for which RTT permits will be issued by the RC till the approval of said guidelines.
2. All such applicants required to submit the Form-I, NJSP, shelf life and packaging data of technical for its registration.
3. After the approval of the guidelines the RTT permits shall be issued accordingly.
4. The application for technical and formulation registration with prescribed requirements of the approved guidelines shall be submitted simultaneously.

Guidelines for formulation under import/indigenous manufacturing with registration of technical (first time registration of a molecule). The data requirement for first time registration of technical (These data recruitment has to be ful filled by the applicant in case of formulation import/ indigenous manufacturing with registration of technical)

Abbreviations :					
R	:	Required	NR	:	Not Required
TIM	:	Technical Indigenous Manufacture	TI	:	Technical Import

Note:
(i) Data requirements of chemical pesticides are also applicable for registration of chemical plant growth regulators (PGR).

(ii) The data requirement for formulation under import and formulation indigenous manufacturing (New molecule remains same as prescribed by the RC in its 284th RC meeting).

S. No.	Parameter	9(3B)		9(3)	
A.	Chemistry	TI	TIM	TI	TIM
1.	Source of Supply of Technical	R	NR	R	NR
2.	Chemical Composition	R	R	R	R
3.	Documents such as registration certificate containing registered CC in the country of Import	R	NR	R	NR

4.	Certificate of DNA	R	NR	R	NR
5.	Chemical Identity of technical	R	R	R	R
6.	Physico - Chemical Properties of adjuvant	R	R	R	R
7.	Technical Bulletin	R	NR	R	NR
8.	Product Specification & Method of analysis for AI and its impurities	R	R	R	R
9.	Analytical Test Report	R	R	R	R
10.	Identification& Quantification of identifiable Impurities	R	R	R	R
11.	Shelf-life claim	R	R	R	R
12.	Shelf-life Data	NR	NR	R	R
13.	Establishment of Chemical Equivalence	NR	NR	NR	NR
14.	Process of Manufacture in detail from 1st step	R	R	R	R
15.	Information about Raw Materials Used and their formula	R	R	R	R
16.	Their Source of Supply.	R	R	R	R
17.	Step-wise Manufacturing Process with condition.	R	R	R	R
18.	Chemical Equation and Formula	R	R	R	R
19.	Flow sheet diagram of process of manufacture	R	R	R	R
20.	Effluent Treatment method	NR	R	NR	R
21.	Certificate of manufacturing license if issued or any other approval under any Govt. regulation to support that applicant is a manufacturer.	R	NR	R	NR
22.	Certificate from manufacturer that the dealer/ trader is an authorized dealer/ trader of the manufacturer.	R	NR	R	NR
23.	A test report about the quality of the product from a laboratory as per GLP scheme. This requirement will be provided	R	NR	R	NR

	along with first consignment. Thereafter, each consignment should have proper analytical test report of the manufacturer.				
24.	In process sample to be drawn of technical u/s 9(3) TIM. In case of technical grade pesticides u/s 9(3), along with drawl of samples, std. of impurities are also to be provided for chemical verification.	R	R	R	R
25.	Methodology for residue estimation as per BIS format.	R	R	R	R
B.	BIOEFFICACY				
1.	Bio-effectiveness	R$^{\$}$	R$^{\$}$	R$^{\$}$	R**
2.	Phytotoxicity	R$^{\$}$	R$^{\$}$	R$^{\$}$	R**
3.	Effect on parasites & predators	R$^{\$}$	R$^{\$}$	R$^{\$}$	R**
4.	Translocation in plants	R	R	R	R
5.	Metabolism in soil	R	R	R	R
6.	Metabolism in water	R	R	R	R
7.	Metabolism in plant	R	R	R	R
8.	Persistence in soil	R	R	R	R
9.	Persistence in water	R	R	R	R
10.	Persistence in plant	R	R	R	R
11.	Compatibility with other chemicals	NR	NR	R$^{\#}$	R$^{\#}$
12.	Residues in plant	NR	NR	R	R
13.	Residues in soil	NR	NR	R	R
14.	Residue tolerance limits fixed by foreign countries	NR	NR	R	R
15.	Cost benefit ratio	NR	NR	R	R
16.	Registration status in foreign countries	R	NR	R	NR

	R$: Two seasons/years data generated at minimum two agroclimatic conditions/locations+ R**: Two seasons/years data generated at minimum three agroclimatic conditions/locations+ Locations+ : Locations shall be applicable for the crops for which required agro-climatic conditions are not availble. R #: Data on compatibility is required, if the product is proposed to mix with other chemicals. Notes: (i) 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 1, green gram at location 2, sesamum at location 3, 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.)* (i) For the requirement of data on translocation in plants, International data from any authentic source shall be accepted.

C. TOXICITY

1.	Acute oral in rat & mice	R	R	R	R
2.	Acute dermal	R	R	R	R
3.	Acute inhalation	R	R	R	R
4.	Primary skin irritation	R	R	R	R
5.	Irritation to mucous membrane	R	R	R	R
6.	Sub-acute oral rat	R	R	R	R
7.	Sub-acute oral dog	R*	R*	R*	R*
8.	Sub-acute dermal	R	R	R	R
9.	Sub-acute inhalation	R	R	R	R
10.	Neuro-toxicity	NR	NR	R	R

11.	Synergism & potentiation	NR	NR	R	R
12.	Teratogenicity	NR	NR	R	R
13.	Effect on reproduction	NR	NR	R	R
14.	Carcinogenicity	NR	NR	R	R
15.	Metabolism	NR	NR	R	R
16.	Mutagenicity	NR	NR	R	R
17.	Toxicity to birds (two)	R	R	R	R
18.	Toxicity to fish (fresh water)	R	R	R	R
19.	Toxicity to honeybees	R	R	R	R
20.	Toxicity to live stock	R	R	R	R
21.	Medical data	R	R	R	R
22.	Human toxicity information from foreign countries	R	R	R	NR
23.	Observation in man (Health records of spray operators)	NR	NR	NR	NR
24.	Health records of Industrial workers.	NR	NR	R	NR
25.	Toxicity to live stock (Field trial & observation)	NR	NR	NR	NR
26.	International report on carcinogenicity & genotoxicity status	NR	NR	R/NR	R/NR
	NR^^: Not required as per decision of 318th RC held on 27-04-2011 **NF** :** In case of wetable powder, if toxicological data is generated for EC formulation applicable as per guidelines, then there is no need to generate data on wettable powder containing the same a.i. **R***: Any peer reviewed published data/information shall be acceptable (approved in 343rd and 344th RC Meeting)				
D. PACKAGING					
1.	Labels and Leaflets 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	R	R	R	R

	(Ultra small, small or Big whichever is applicable)				
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

Annexure-10.32.2

GUIDELINES / DATA REQUIREMENTS FOR GRANT OF REGISTRATION OF PESTICIDES UNDER VARIOUS CATEGORIES:

FOR REGISTRATION OF INSECTICIDES / PESTICIDES UNDER SECTION 9(3B), 9(3) AND 9(4) OF THE INSECTICIDES ACT, 1968 AND THE RULES 1971.

GUIDANCE TO STAKEHOLDERS:

A. Stakeholders generating data and submitting the application for registration should ensure that the tests are conducted and data generated in accordance with established scientific procedures following the test guidelines and the principles of Good Laboratory Practices, wherever applicable. The data should be authentic,replicable, utilizable and of good quality. **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 (MCPA) or Botanical or Semio-chemical/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, SC etc.) or Vapor (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)] as per the provisions (Please refer section (9) of the Insecticides Act, 1968); and 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.**

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**

ANNEXURE-I
LEGAL REQUIREMENTS

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 or other legal agreement of manufacturing site purported to be used as manufacturing site**(Notarized)**
8. Technical to be used in formulation should be duly registered in India or the registration application of the Technical should be submitted in parallel to formulation registration application with necessary references (In case of 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/apostle documentsin 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 & Manufacturing License obtained and products actually manufactured during the previous 3 (three) years ***(Applicable in case of 9(4) TI/FI & FIM Applications.***

Note:The RC has decided to implement the KYC as per decision taken in its 424th meeting. As per requirement the module has been brought to working condition. The applicant are supposed to feed their documents are per requirement.
If, the "KYC" is activated, then the applicant needs not to submit the following documents repeatedly:

1. Copy of BOD Resolution/Affidavit/Partnership deed (**Notarized**)
2. Certificate as per category of Industry/ Manufacturing license (**Notarized**)
3. PAN No. (**Notarized**)
4. Incorporation Certificate (**Notarized**) and other documents as per KYC requirement
5. Proof of ownership/lease agreement of manufacturing site purported to be used as manufacturing site**(Notarized)**
6. List of products for which the registration has been given to the firm and Manufacturing License obtained and products actually manufactured during the previous 3 (three) years.

ANNEXURE-II
DATA REQUIREMENTS FOR CHEMICAL PESTICIDES

A. Chemistry:

Sl. No.	Parameter	9(3B)			9(3)				9(4)			
		TI	TIM	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.	Details of 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 adjuvant 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 BIS format/BIS No. if published	R	R	R	R	R	R	R	NR	R*	NR	NR
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	R	R	R	R	R	R	R	NR	NR	NR	NR

	meteorological data for corresponding period											
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 registeredthe Technical Grade Pesticide/ Insecticide /Formulation and that the consents supplying the Technical Grade Pesticide/ Insecticide/ Formulation to the applicant.	R	NR	R	R	NR	R	R	R	NR	R	NR
17.	Registration Certificate from Designated National Authority (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	R	NR	NR	R	NR	R	NR	R	NR	R	NR

	approved source of import (actual manufacturer).Or Principal company or Subsidiary of registrant of pesticide in India or the supplier authorized by manufacturer and mention the full details of the source of import in the invoice.											
19.	An In-Process sample to be drawn from the R&D/manufacturing facility of applicant in case of technical indigenous manufacture of the insecticide/sample to be submitted in case of technical import[$]; 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 and FI category sample and CRM to be submitted in CIL Faridabad.	R	R	R	R	R	R	R	NR	R	NR	NR
20.	Methodology for residue estimation in BIS format.	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.
4. Sample shall be drawn in case of 9(3) TIM and 9(4) TIM as per approved procedure in 442ndRC or as and when amended by RC.In case of 9(3)TI5 batch GLP analysis report to be submitted.

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 oilnatural mineral oil products shall be for 9(3b) and 9(3) category only.
10. 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 required to submit the data as per the modalities and guidelines approved for the said purpose and available at annexure 10.32.1, for registration of technical.
11. 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)
12. In case of formulation of pre-mix combination product having three active ingredients, preferably a single method of analysis must be used, otherwise applicant should be submitted technical justification for using more than one method of analysis.

Modalities and Guidelines for formulation under import / indigenous manufacturing with registration of technical (first time registration of a molecule) as approved vide annexure 10.32.1 -reg.

The proposed requirements in the guidelines as mentioned below and the proposed modalities of its implementations are as under:

5. The guidelines once approved shall be applicable for the insecticide/pesticides for which RTT permits will be issued by the RC till the approval of said guidelines.
6. All such applicants required to submit the Form-I, NJSP, shelf life and packaging data of technical for its registration.

7. After the approval of the guidelines the RTT permits shall be issued accordingly.
8. The application for technical and formulation registration with prescribed requirements of the approved guidelines shall be submitted simultaneously.

Guidelines for formulation under import/indigenous manufacturing with registration of technical (first time registration of a molecule). The data requirement for first time registration of technical (These data recruitment has to be ful filled by the applicant in case of formulation import/ indigenous manufacturing with registration of technical)

Abbreviations :					
R	:	Required	NR	:	Not Required
TIM	:	Technical Indigenous Manufacture	TI	:	Technical Import

Note:
(i) Data requirements of chemical pesticides are also applicable for registration of chemical plant growth regulators (PGR).
(ii) The data requirement for formulation under import and formulation indigenous manufacturing (New molecule remains same as prescribed by the RC in its 284th RC meeting).

S. No.	Parameter	9(3B)		9(3)	
E.	Chemistry	TI	TIM	TI	TIM
26.	Source of Supply of Technical	R	NR	R	NR
27.	Chemical Composition	R	R	R	R
28.	Documents such as registration certificate containing registered CC in the country of Import	R	NR	R	NR
29.	Certificate of DNA	R	NR	R	NR
30.	Chemical Identity of technical	R	R	R	R
31.	Physico - Chemical Properties of adjuvant	R	R	R	R
32.	Technical Bulletin	R	NR	R	NR
33.	Product Specification & Method of analysis for AI and its impurities	R	R	R	R
34.	Analytical Test Report	R	R	R	R
35.	Identification& Quantification of identifiable Impurities	R	R	R	R
36.	Shelf-life claim	R	R	R	R

37.	Shelf-life Data	NR	NR	R	R
38.	Establishment of Chemical Equivalence	NR	NR	NR	NR
39.	Process of Manufacture in detail from 1st step	R	R	R	R
40.	Information about Raw Materials Used and their formula	R	R	R	R
41.	Their Source of Supply.	R	R	R	R
42.	Step-wise Manufacturing Process with condition.	R	R	R	R
43.	Chemical Equation and Formula	R	R	R	R
44.	Flow sheet diagram of process of manufacture	R	R	R	R
45.	Effluent Treatment method	NR	R	NR	R
46.	Certificate of manufacturing license if issued or any other approval under any Govt. regulation to support that applicant is a manufacturer.	R	NR	R	NR
47.	Certificate from manufacturer that the dealer/ trader is an authorized dealer/ trader of the manufacturer.	R	NR	R	NR
48.	A test report about the quality of the product from a laboratory as per GLP scheme. This requirement will be provided along with first consignment. Thereafter, each consignment should have proper analytical test report of the manufacturer.	R	NR	R	NR
49.	In process sample to be drawn of technical u/s 9(3) TIM. In case of technical grade pesticides u/s 9(3), along with drawl of samples, std. of impurities are also to be provided for chemical verification.	R	R	R	R
50.	Methodology for residue estimation as per BIS format.	R	R	R	R
F.	BIOEFFICACY				
17.	Bio-effectiveness	R$	R$	R$	R**

18.	Phytotoxicity	R$^{\$}$	R$^{\$}$	R$^{\$}$	R**
19.	Effect on parasites & predators	R$^{\$}$	R$^{\$}$	R$^{\$}$	R**
20.	Translocation in plants	R	R	R	R
21.	Metabolism in soil	R	R	R	R
22.	Metabolism in water	R	R	R	R
23.	Metabolism in plant	R	R	R	R
24.	Persistence in soil	R	R	R	R
25.	Persistence in water	R	R	R	R
26.	Persistence in plant	R	R	R	R
27.	Compatibility with other chemicals	NR	NR	R$^{\#}$	R$^{\#}$
28.	Residues in plant	NR	NR	R	R
29.	Residues in soil	NR	NR	R	R
30.	Residue tolerance limits fixed by foreign countries	NR	NR	R	R
31.	Cost benefit ratio	NR	NR	R	R
32.	Registration status in foreign countries	R	NR	R	NR

R$^{\$}$: Two seasons/years data generated at minimum two agroclimatic conditions/locations+

R**: Two seasons/years data generated at minimum three agroclimatic conditions/locations+

Locations+ : Locations shall be applicable for the crops for which required agro-climatic conditions are not availble.

R #: Data on compatibility is required, if the product is proposed to mix with other chemicals.

Notes:

(i) 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 1, green gram at location 2, sesamum at location 3, 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.)* (ii) For the requirement of data on translocation in plants, International data from any authentic source shall be accepted.				
G.	TOXICITY				
27.	Acute oral in rat & mice	R	R	R	R
28.	Acute dermal	R	R	R	R
29.	Acute inhalation	R	R	R	R
30.	Primary skin irritation	R	R	R	R
31.	Irritation to mucous membrane	R	R	R	R
32.	Sub-acute oral rat	R	R	R	R
33.	Sub-acute oral dog	R*	R*	R*	R*
34.	Sub-acute dermal	R	R	R	R
35.	Sub-acute inhalation	R	R	R	R
36.	Neuro-toxicity	NR	NR	R	R
37.	Synergism & potentiation	NR	NR	R	R
38.	Teratogenicity	NR	NR	R	R
39.	Effect on reproduction	NR	NR	R	R
40.	Carcinogenicity	NR	NR	R	R
41.	Metabolism	NR	NR	R	R
42.	Mutagenicity	NR	NR	R	R
43.	Toxicity to birds (two)	R	R	R	R
44.	Toxicity to fish (fresh water)	R	R	R	R
45.	Toxicity to honeybees	R	R	R	R

46.	Toxicity to live stock	R	R	R	R
47.	Medical data	R	R	R	R
48.	Human toxicity information from foreign countries	R	R	R	NR
49.	Observation in man (Health records of spray operators)	NR	NR	NR	NR
50.	Health records of Industrial workers.	NR	NR	R	NR
51.	Toxicity to live stock (Field trial & observation)	NR	NR	NR	NR
52.	International report on carcinogenicity & genotoxicity status	NR	NR	R/NR	R/NR
	NR^^: Not required as per decision of 318th RC held on 27-04-2011 **NF** :** In case of wetable powder, if toxicological data is generated for EC formulation applicable as per guidelines, then there is no need to generate data on wettable powder containing the same a.i. **R***: Any peer reviewed published data/information shall be acceptable (approved in 343rd and 344th RC Meeting)				
H. PACKAGING					
9.	Labels and Leaflets per IR-1971, all fields (as applicable) and as amended from time to time	R	R	R	R
10.	Manner of labeling and Leaflet	R	R	R	R
11.	Type of packaging (Ultra small, small or Big whichever is applicable)	R	R	R	R
12.	Manner of packaging	R	R	R	R
13.	Specification for primary, Secondary and Transport packages (whichever is applicable)	R	R	R	R
14.	Details of packaging material and its compatibility with content	R	R	R	R
15.	Performance of container with content during storage stability test(Shelf life Study)	R	R	R	R
16.	Transport worthiness test	R	R	R	R

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

Table-1

Sr. No.	Parameters	9(3b)			9(3)					9(4)
		TI	**TIM**	**FIM**	**TI**	**TIM**	**FI**	**FIM**	**Label expansion**	**TI/TIM/FI/FIM or endorsement of already approvedlabel 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 natural enemies (parasitoids and predators) (Insecticides)	NR	NR	R*	NR	NR	R**	R**	R**	
5.	Effect on beneficial soil micro-organisms &physico-chemical properties (Herbicide only). Effect on beneficial soil micro-organisms in case of Seed treatment and on soil applied pesticides (Fungicides, Insecticide and PGRs).	NR	NR	R*	NR	NR	R**	R**	R**	
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	NR	R	R	NR	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 or 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	
19.	MRL Performa along with Pen drive in duplicate (other than seed treatment).	NR	NR	R	NR	NR	R	R	R	
20.	Label and Leaflets (as per Insecticides Rule 18 and 19)	R	R	R	R	R	R	R	R	

Remarks on Bio-efficacy 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 conditions.

c. **R****: Two seasons/years data generated at minimum three different agro-climatic conditions

d. **R#:** One season/year data generated at minimum four different agro-climatic conditions 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 conditions in case of 9(3).** In case of seed treatment one season / year data generated at minimum three different agro-climatic conditions 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 suffice.

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

e. **R$:** If Technical Import & Technical Indigenous Manufacture application which are submitted together with Formulation Import & FormulationIndigenous 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 sesame 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 / FormulationIndigenous Manufacture. However, data on Sr. No. 1, 2 (if any individualcomponent 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 formulationsof individual component of the formulation at approved dosage shall also be included as check), 4, (required only for insecticides {Natural enemy data not applicable for PGRs})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) bio-efficacy 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 (Waiver on residue/persistence studies can be accepted vide 313rd RC Meeting Decisions) and/or any adjuvants/diluents (other than water)/carrier is different that are in the 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 than 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 conditions is required and one seasondata on Sr. No. 14 (Table – 1) on two representative crops particularly on fruits and vegetables is required. For registration of technical bio-efficacy data will not be required where formulation folder with bio-efficacy data is submitted for registration. However, if application is submitted for technical alone then chemical equivalence (through five batch analysis) with already registered technical is required to get this exemption (As approved in 427 RC meeting vide agenda item no. 3.2).Modalities of 5 batch analysis are as per 447th RC approval.

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 one representative registered formulations with highest a.i. content 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 bio-efficacy 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 Bio-efficacy 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 bio-efficacy 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 Bio-efficacy 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 Bio-efficacy will be required except Registration status of MUP in foreign countries.
   c. Rationale for import and registering the MUP to be submitted in Bio-efficacy.

9. **For use of Surfactant with registered pesticide Formulations,** data ontwo 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 onSr. 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 bio-efficacy 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 in the range of 15-20% (on disease basis) during the trial period and the fungicide should show minimum 60-70% 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 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 (for gases and fumigants)

(iii) Fate and behavior in water (for solid and liquid pesticides)

(iv) Fate and behavior in soil (for solid and liquid pesticides) and 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 427thRC 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) (**except for Ethephon (formulation) for use as ripening agent as per directions of Hon'ble Delhi High court in W.P. (C) No. 1987 of 2022)** are as under**: -**

Table – 2

1.	Data on bio-effectiveness Adverse effects on postharvest crop produce, if any (phyto-toxicity, change in appearance and flavour etc.)	For label claim of controlled atmospheric conditions data on 1. And 2. To be submitted for threerepeated trials (same temperature and relative humidity) OR For label claim of ambient atmospheric conditions data to be submitted for minimum three repeated trials, regardless of season.
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. OR The requirement of one season four locations will be applicable as per insecticide, fungicide and PGR guidelines.
3.	Registration status of formulation in other countries	Required
4.	MRL Performa and label leaflets	Required
5.	Label claims as per product Bio-efficacy information as per gazette notifications issued by Govt. of India from time to time.	

Guidelines for import and indigenous manufacture of Ethephon (formulation) for use as ripening agent. (As approved in 447RC meeting vide agenda item no. 10.51)

Sl. No.	**Parameter**	**9(3)**	
		FI	**FIM**
1	**2**	**3**	**4**
A.	**CHEMISTRY**		
1	Chemical composition of formulation.	R	R
2	Chemical identity of technical and adjuvants	R	R
3	Physico-chemical properties of technical and adjuvants	R	R
4	Specification of the product and Method of analysis	R	R
5	Analytical Test report	R	R
6	Data on rate of release/application	R	R
7	Shelf life claim & data	R	R
8	Process of manufacture, Step-wise manufacturing process and flow sheet diagram of process of manufacture	R	R
9	Information about raw material used and their source of supply	R	R

10	The applicant should provide sample alongwith ref./analytical standard for pre-registration verification along with Specification, Method of analysis, Chromatograms etc.		R	R
11	Methodology for residue estimation as per BIS format		R	R
12	Source of supply of technical: The technical should be registered in India for use in country and proof of supply that the formulated product has been manufactured using the particular technical only for consent provided		R	R
13	Form-I, Labels/Leaflets and copy of RTT permit, if relevant.		R	R
14	ETP certificate, if applicable		R	R
B.	**BIO-EFFICACY & RESIDUES**		**FI**	**FIM**
1	Data on bio-effectiveness.	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 condition data to be submitted form three repeated trials Of different locations.	R	R
2	Adverse effect on post-harvest crop produce, if any(phytotoxicity, change in appearance and flavor etc.)		R	R
3	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 trails. Or For label claim of ambient atmospheric condition data to be submitted form three repeated trials of different locations.	R	R
4	Registration status of formulation in other countries		R	R
5	MRL Performa and label leaflets		R	R
6	Label claims as per product Bio-efficacy : Information as per latest Gazette Notification issued by the Government of India		R	R
C.	**TOXICITY**		**FI**	**FIM**
1	Acute Oral toxicity in rat		R	R
2	Acute dermal toxicity		R	R
3	Acute inhalation toxicity		R	R
4	Primary skin irritation in rabbit		R	R
5	Irritation to mucous membrane in Rabbit		R	R
6	Skin sensitization test		R	R
7	Observation in man if the concentration is lower and WP in place of EC etc., data may not be required (NR type of formulation is such i.e. as per decision of 318th RC held on 27.04.2011)		R	R

8	Toxicity to birds (One species only)	R	R
9	Medical data	R	R
	Note: If the formulation is more toxic than its technical, then the toxicity data on sub-acute oral rat, sub-acute dermal, sub-acute inhalation, neurotoxicity, sub-acute oral dog (Any peer reviewed published data/information for sub-acute oral dog only as approved in 343 and 344 RC meeting) will be acceptable.		
D.	**PACKAGING**	**FI**	**FIM**
1	**Labels and leaflets as per-1971 existing norms**		
a	For size 250 ml/gm & below	R	R
b	For 500 ml/gm & above	R	R
2	**Label to contents**		
a	Detailed Chemical composition	R	R
b	Purpose for import/manufacture	R	R
c	Antidote	R	R
d	Toxicity triangle	R	R
e	Cautionary statement	R	R
f	Brief direction concerning usages	R	R
g	Restriction if any	R	R
3	**Leaflets to contain:**		
a	Detailed chemical composition leaflets accompanying small labels (upto 250mg/ml size container)	R	R
b	Introductory para about the pesticide	R	R
c	Detailed directions concerning usages	R	R
d	Time of application	R	R
e	Application equipment	R	R
f	Waiting period	R	R
g	Symptoms of Poisoning	R	R
h	First-aid measures	R	R
i	Antidote and treatment	R	R
j	Restriction, if any	R	R
k	Instruction for storage	R	R
l	Information regarding disposal of used packages	R	R
4	**Type of packaging (packing material + compatibility with content)**	R	R
5	**Manner of packing**		
a	Specification for primary package	R	R
b	Specification for secondary packaging	R	R
c	Specification for transport packaging	R	R
d	Manner of labeling	R	R
e	Performance of container during storage stability test	R	R
f	Transport worthiness Test	R	R
E	**LEGAL**	**FI**	**FIM**
1	Copy of BOD Resolution/ Affidavit / partnership deed.{notarized)	R	R
2	Affidavit for Chemical composition on NJSP (Notarized)	R	R
3	Certificate as per category of industry/ Manufacturing license (notarized)	R	R
4	PAN No. (notarized)	R	R

5	Incorporation Certificate (Notarized)	R	R
6	Proof of ownership of industry plot	R	R
7	Proof of Source of Technical to be used in formulation is duly registered. (ln 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].	R	R
8	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)	R	R
9	Copy of 9(3b) Registration certificate, if relevant	R	R

Remarks- 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 (Data must be generated as per ICMR protocols). 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 onEffect 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.
3. Bio-efficacy and residue data:Bio-efficacy test on the proposed formulation should be conducted in Indian conditions minimum 02 trials in each of 3 National Laboratories / NABL accredited laboratories, recognized by Government of India.
4. 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. Bio-efficacy data generated by ICMR / MOH&FW Institutes based on multi-centric three years /seasons as per their protocol.
2. For WHOPES recommended insecticides, Phase I data is exempted (Data must be generated as per ICMR protocols).

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 equipment 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 Bio-efficacy 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 pest 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-Rat	R	R	R	R	NR	NR
4.	Primary Skin Irritation-Rabbit	R	R	R	R	NR	NR
5.	Acute Eye Irritation-Rabbit	R	R	R	R	NR	NR
6.	Skin Sensitization Test – Guinea Pig	R	R	R	R	NR	NR
7.	Repeated dose range finding oral toxicity study (28 days)*-Rat	R	R	NR	NR	NR	NR
8.	Repeated dose 90 days oral (Rat)	R	R	NR	NR	NR	NR
9.	Repeated dose 90 days oral toxicity-Dog***	R	R	NR	NR	NR	NR
10.	Repeated dose dermal toxicity-Rat/Rabbit	R	R	NR	NR	NR	NR
11.	Repeated dose inhalation toxicity*#-Rat	R	R	NR	NR	NR	NR
12.	Acute Neuro-toxicity-Rodent*	R	R	NR	NR	NR	NR
13.	Repeated dose Neurotoxicity-Rodent*	R	R	NR	NR	NR	NR
14.	Delayed Neurotoxicity- OP compound- Acute exposure-Laying Hen	R	R	NR	NR	NR	NR
15.	Delayed neurotoxicity-OP compound-Repeated Administration-Laying Hen	R	R	NR	NR	NR	NR

16.	Developmental Neurotoxicity*-Rodent	R	R	NR	NR	NR	NR
17.	Combined carcinogenicity & chronic toxicity-Rat	R	R	NR	NR	NR	NR
18.	Carcinogenicity-Mice	R	R	NR	NR	NR	NR
19.	Developmental toxicity study- a) Rat & b) Rabbit	NR	R	NR	NR	NR	NR
20.	Two generation reproduction toxicity in Rat	NR	R	NR	NR	NR	NR
21.	Mutagenicity **	NR	R	NR	NR	R	NR
22.	Pharmacokinetics and Metabolism in Rat.	R	R	R	R	NR	NR
23.	Feeding study including Metabolism in Livestock (Goat, Cow) / Poultry (Hen)	R	R	NR	NR	NR	NR
24.	Pharmacokinetics and Metabolism in other mammals and its similarities or differences from humans.	NR	NR	NR	NR	NR	NR
25.	Immuno-toxicity study*	R	R	NR	NR	NR	NR
26.	Acute Avian toxicity *	R	R	R	R	NR	NR
27.	Repeated dose Avian toxicity (One species)	R	R	NR	NR	NR	NR
28.	Avian Reproduction toxicity (One species)	R	R	NR	NR	NR	NR
29.	Acute toxicity to Fresh Water Fish (One species)	R	R	R	R	NR	NR
30.	Acute Toxicity-Honey Bee (Oral & Contact)	R	R	R	R	NR	NR

31.	Acute Toxicity-Earthworm	R	R	R	R	NR	NR
32.	Medical data	R	R	R	R	NR	NR
33.	Human toxicity information from foreign countries	NR	NR	NR	R	NR	NR
34.	Health records of Industrial workers	NR	R	NR	R	NR	NR
35.	International report on Carcinogenicity & Genotoxicity	NR	NR	NR	NR	NR	NR

Footnotes:

Toxicity study No. 9- ***Repeated dose 90 days oral toxicity study (Dog): Studies, as per the guidelines, will be required for new molecules. However, peer reviewed internationally published data/ literature is also acceptable for such studies.

Toxicity study no. 17,18

i. The Combined Carcinogenicity and Chronic Toxicity study in Rat and

ii. Separately carcinogenicity study in mice is required.

Toxicity study no. 23-Feeding and metabolism study can be done together.

Some metabolites can be more harmful than parent compound even in very small quantity due to bio-activation so it can be conditional requirement, depending on 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 35 are required.

   b. **TIM u/s 9(3):-**In case of Technical Indigenous Manufacture, data of Sl. No. 33 and S. No. 34 are not required.

3) **Technical Import from new Source u/s 9(3):-**Data on parameters 1 to 11;21, 32-33 and 35 is required.

4) **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.

5) **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.**

6) **9(4) TIM:-**In case of Technical indigenous Manufacture u/s 9(4), data on Sl. No. 21 data only AMES test is required.

7) **Formulation u/s 9(3b):** Data as per FIM u/s 9(3b) required as indicated in table above.

8) **Formulation u/s 9(3):-**For FIM :- as indicated in table except data of Sl. No. 33 and S. No. 34 are not required.

9) **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 , orTIM (as the case may be) should also be submitted.

10) **9(4) TI/FIM:-**No data is required, in case of Technical Import(TI) or Formulation Indigenous Manufacture (FIM) u/s 9(4) application.

11) **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).

12) **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), Bio-efficacy, Toxicity and Packaging as per the applicable guidelines for registration of technical should be submitted.

13) **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.

14) **Pesticide Formulation for use in Public Health:** Data on the parameters from S. No. 1 to 6, 26 & 29 should be submitted. Recommendations from National Vector Disease Control Program, M/o Health & Family Welfare are also required.

15) **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.

16) **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.

***** 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.

Note: The general recommendations as mentioned in the Guidance Document on Toxicology for registration of Chemical Pesticides in India will be applicable on the basis of merit case to case basis.

- **Waiver would be considered only when existing information provides robust and full scientifically sound weight of evidence approach and read across/bridging from structurally and /or biologically related similar pesticides specifically case to case basis on full merits.**
- **The replacement alternatives not involving experiments on animals would be accepted in exceptional cases where validated 3Rs (Replacement, Reduction and Refinement) methods recognized by OECD and other renowned Regulatory Authorities is provided on case to case basis.**
- **As per the decision of 437th RC, if the formulation is more toxic than its technical, then the toxicity data on sub-acute oral rat, sub-acute dermal, sub-acute inhalation, neurotoxicity and development toxicity will be required.**

D. PACKAGING

Sl. No.	Parameter	Section 9(3B)			Section 9(3)				Section 9(4)			
		TIM	FIM	TI	TIM	FIM	TI	FI	TIM	FIM	TI	FI
1.	Labels and Leaflets per IR-1971, all fields (as applicable) and as	R	R	R	R	R	R	R	NR	NR	NR	NR

	amended from time to time											
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	R	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)and manner of packaging 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 54^0C ± 2^oC (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, **ifapplicable.**
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 partnershipfirms.
7. Correct composition as per earlier 9(3) / 9(3B) registrant of bio-pesticidestrain.
8. The applicant should also, submit notarized copy of the Permanent Account No. (PAN), allotted by the Income TaxDepartment.
9. In case of company, the Certificate of Incorporation granted by the Registrar ofCompanies.
10. Fee receipt issued by NBAIM, MaunathBhanjan, 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) Entomo-pathogenic/ Entomo-toxicBacteria
ii) AntagonisticBacteria
iii) Entomo-pathogenicFungi
iv) AntagonisticFungi
v) Nuclear Polyhedrosis Virus (NPV) & Granulosis Virus (GV)

A. Chemistry:

Sl. No.	Characteristics	Microbial (Antagonistic bacteria, Entomo-pathogenic/Entomo-toxic bacteria, Entomo-pathogenic fungi, Antagonistic fungi, and Baculovirus			
		Primary/Mother Culture		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, suspensibility, miscibility etc parameters	R	R	R	R
6.	Isolation of strain &Manufacturing process	R	R	R	R
7.	Methods of analysis including Quantitativeanalysis	R*	R*	R*	R*
8.	Shelf life claims	R	R	R	R
9.	Data on storage stability as per shelf lifeclaims	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 endotoxin)	NR#	NR#	NR#	NR#
12.	CFU/g or ml	R	R	R	R

13.	POB/Capsule count per 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/ dispending agent, stabilizers/ emulsifiers, contaminants/ impurities etc.	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 x 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@ g or 500@ mL as the case may be)				
	a. DNA fingerprinting for the strain verification from NBAIM, MaunathBhanjan.	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 Entomo-toxic-bacteria.

- # and $ these parameters are required for Entomo-toxic 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 Entomo-toxic bacteria.
- POB/Capsule count per ml/g of the product only forNPV.
- Viral unit: NPVs $1x10^9$ POB/ml or gm. minimum, GVs: $5x10^9$ 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 rootcolonization.
- Natural occurrence of the organism, Immunology assays: Elisa and Separation and purification of crystals are required for Entomo-toxicbacteria.
- 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 Entomo-pathogenic Viruses. Production of Entomo-pathogenic 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 labor 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 thestrain.

4. Relevant Affidavit/Undertakings:-
   a) Affidavit on bio-pesticide composition on NJSP dulynotarized.
   b) Notarized copy of depositing microbial bio-pesticides strain sample in National Repository with reference codenumber.
   c) Undertaking on NJSP duly notarized that product do not contain any genetically modified organism in the prescribedformat.
   d) Undertaking on NJSP duly notarized that product is free fromchemical/botanical pesticides / other agro-chemicals.

e) Affidavit of strain Innovator orapplicant
f) Copy of 9(3B) Registration certificate, ifrelevant.

Note:

1. Bt products should be labeled 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 thecrystal.
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 *Trichoplusiani*/ *Helicoverpaarmigera*for Lepidopteran specific Bt formulations. *Spodoptera* Units (SPU), *Leptinotarsa* Units (LTUs) or International Toxin Units (ITUs) are to be used for denoting a specificinsect.
4. No test for beta exotoxin is required for *Bacillus sphaericus*, because this species is not known to produceexotoxins.
5. The bio-potency 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/mgpowder.
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 pippins (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 pp45).
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 alternativepowders/strains.
8. Water content should not exceed 8 %, (12% in *Pseudomonas spp*) to preclude premature degradation of theproduct.

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 partnershipfirms.

III. Correct composition as per earlier 9(3) registrant of bio-pesticidestrain.

IV. The applicant should also, submit notarized copy of the Permanent Account No. (PAN), allotted by the Income TaxDepartment.

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 stamp and envelopes.

VIII. Copies of Label Leaflets of the product as approved by RC of already registeredstrain.

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 gm/ml quantity shall be deposited to NBAIM, MaunathBhanjan 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 oronline.

XII. Original fee receipt issued by NBAIM, MaunathBhanjan,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 bio-pesticide registration, henceforth. The applications under scrutiny in the Secretariat of CIB&RC are also covered under thisdecision.

XVI. A sample of 500 gm/ml shall be deposited in the Secretariat of CIB&RC along with File/documents for PRVpurposes.

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 orotherwise.

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 fromRC.

Annexure-I

AFFIDAVIT ON BIO-PESTICIDE STRAIN BY INVENTOR OR APPLICANT

I, …….. S/o ……, aged …….years, resident of ……..and 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/fourmonths.
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/ CONTAMINATS/OTHER AGRO-CHEMICALS;

I, …….. S/o ……, aged …….years, resident of ……..and Proprietor/Authorized person of the firm M/s ……., having its office at …….. do hereby declare and solemnly affirms asunder:

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 ……, aged …….years, resident of ……..and Proprietor/Authorized person of the firm M/s ……., having its office at …….. do hereby declare and solemnly affirms asunder:

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 1x108 CFU/gm min1.0%
b)	Carboxy methyl cellulose	:	1.0%
c)	TalcPowder	:	98.0%
	Total	:	100.0%

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

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 orNil.

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 therefrom.

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----------basedon------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 thisproduct.

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-pesticidesoftheInternationalPlantProtectionConvention(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 forverification.

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, Directorate 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 fromthem.

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

Signature:----------

Date------- Name----------

Place:---------- 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 phyto-toxicity generated at ICAR, SAUs, CSIR / ICMR institutes. The data should be certified either by the Director or Head of theInstitute.	NR	NR	R**	R***
2	**Laboratory studies:** The product should be tested at a laboratory under ICAR/ SAU/ CSIR/ICMR. **2.1)** LC_{50} values for each insect species under laboratory conditions should be generated at least at two institutes of ICAR, SAUs, CSIR andICMR. **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 Entomo-toxic Bacteria**
- **– Applicable for NPV & GV.**
- **Sr. No. 3 - Required in case of Entomo-pathogenic fungi, Entomo-pathogenic 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, Entomo-pathogenic/-Entomo-toxic bacteria, Entomo-pathogenic fungi, Antagonistic fungi and Baculo-virus			
		Primary culture/mother culture		Formulated product	
		9(3b)	9(3)	9(3b)	9(3)
1.	**SingleDose Oral – Rat** (Toxicity/Infectivity/Pathogenicity)	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 Intra-peritoneal–Rat (c)** (Toxicity/Infectivity/Pathogenicity)	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 Species)
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 entomo-pathogenic/entomo-toxic bacteria
b. Pulmonary toxicity study required for registration of antagonistic bacteria, antagonistic fungi, entomo-pathogenic fungi,baculo-virus
c. Intra-peritoneal toxicity study required for registration of antagonistic fungi, entomo-pathogenic fungi, antagonisticbacteria
d. Cell culture and Intravenous study required for registration of baculo-virus. e and f – required for all except antagonistic fungi
e. required for all except entomo-pathogenic/entomo-toxicbacteria

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 (minimum 98%) 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.

Note: 1) The general recommendations as mentioned in the Guidance document on Toxicology for registration of chemical pesticides in India will also be applicable for registration of Microbial Pest Control Agents (MPCA) Pesticides on merit case to case basis.

2) Waiver would be considered only when existing information provides robust and full scientifically sound weight of evidence approach and read across/bridging from structurally and /or biologically related similar pesticides specifically case to case basis on full merits.
3) The replacement alternatives not involving experiments on animals would not be normally considered, except in exceptional and rare cases where in case of alternatives if available with full and sound justification is provided specifically case to case basis on merit subject to full satisfaction of the expert regarding full toxicity data provided for the alternatives.

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**	Primary culture/mother culture		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 lifeStudy)	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) and manner of packagingis 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 andapplicable).
3. All Packaging tests must be carried out with the product of same batch and in its commercial package preferably in Indiancondition.
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 storageetc.
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 thescope.
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.

Guidelines on Consortium of Bio-pesticides

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

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

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 are required.

Note:

a. Inhalation toxicity study required for registration of entomo-pathogenic/entomo-toxic bacteria
b. Pulmonary toxicity study required for registration of antagonistic bacteria, antagonistic fungi, entomo-pathogenic fungi,baculo-virus
c. Intraperitoneal toxicity study required for registration of antagonistic fungi, entomo-pathogenic fungi, antagonisticbacteria
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 entomo-pathogenic/entomo-toxic 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 (minimum 98%) 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 (Any new MPCA {Bio-pesticides} other than the list could be developed as Consortia after inclusion in Schedule)

S. No.	Name of the bio-pesticides
1.	*Trichoderma* spp.*(T. viride /T. asperellum, T. harzianum, T. virens etc.)*
2.	*Gliocladium virens T.virens*
3.	*Ampyliomycesquisqualis*
4.	*Coniotyriumminitans*
5.	*Chaetomium globosum*
6.	*Aspergillus niger*(non-pathogenic/biotype)
7.	*Bacillus subtilis*
8.	*Pseudomonas fluorescens, P. protegens, P. entomophila*,
9.	*Streptomyces griseoviridis*
10.	*Streptomyces lidicus*
11.	*Agrobacterium radiobacter*K84
12.	*Beauveria bassiana*
13.	*Metarrhizium anisopliae*
14.	*Nomuraearileyi*, (New name: *Metarhiziumrileyi*)
15.	*Verticillium lecanii*, (New name: *Lecanicilliumlecanii*)
16.	*Verticilium chlamydosporium*, (New name: *Pochoniachlamydosporium*)
17.	*Paecilomyceslilacinus*, (New name: *Purpureocilliumlilacinum*)
18.	*Myrothecium verrucaria-nematicide*
19.	*Bacillus species* (includes *Bacillus sphaericus (syn: Lysinibacillus, 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.	Granulosis Viruses (GV)
21.	Nuclear Polyhedrosis Viruses (NPV) (includes *Spodoptera litura*NPV, *Spodoptera frugiperda*NPV, *Heicoverpaarmigera*NPV, *Spodoptera mauritia*NPV, *Mythimna separata* NPV,

A. INDIAN STANDARDS ANTAGONISTIC FUNGI - DRAFTSPECIFICATIONS

1. Form andappearance
2. pH
3. Composition
   a. CFU/g of theproduct
   b. Percent loading content of the Bio-control organism inthe formulation & nature ofbiomass.
   c. Percentage of carrier/filler, wetting/ dispendingagent,
   d. Stabilizers/ emulsifiers, contaminants/ impuritiesetc.
   e. Moisturecontent
   f. CFU counts: *Trichoderma* $2x10^6$CFU/ml or gm min. (Stability at 30^oC and 65%RH).

4. **Contaminants**:
   a. BiologicalContaminants:
   b. Pathogenic Contaminants: such asgramnegative bacteria*Salmonella,Shigella, Vibrio* etc.:**absent**
   c. Other contaminants should not exceed $1x10^4$/ml org
   d. Chemical/ botanical pesticides contaminants: **absent.**

5. **Method ofanalysis**:
   a. CFU counts by serial dilution and examination under regular compound research microscope with bright fieldoptics.
   b. Plating for contaminants on specificmedia
   c. Antagonistic mycolyticcapability on target organism by bioassay on plants (Laboratorytest).
   d. 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 testpathogen are transferred into the bottle and left for incubation for 15 days. Once the culture hasgrown well, the sand maize medium is mixed along with the fungal growth and 1g from thispreparation is used as the inoculum after adjusting the CFU to 1 x10/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 ¾th 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 $2x10^6$). 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.

B. INDIAN STANDARDS ANTAGONISTIC BACTERIA – DRAFT SPECIFICATIONS

1. Form andappearance
2. pH
3. Composition
   a. Percent content of the Bio-control organism in the formulation & nature ofbiomass
   b. CFU/g or ml of theproduct.
   c. Percentage of other components: carrier /filler, wetting/ dispersing agent, stabilizers/emulsifiers, contaminants/impuritiesetc.
   d. Moisturecontent.
   e. CFU counts: Minimum $1x10^8$ CFU/ml or gm. (Stability at 30^oC and65%RH).
4. Contaminants:
   a. BiologicalContaminants:
   b. Pathogenic Contaminants: such as gram negativebacteria*Salmonella*, *Shigella*, *Vibrio* etc.:**absent**
   c. Other contaminants should not exceed $1x10^4$/ml or g
   d. Chemical/botanical pesticides contaminants: **absent.**
5. Method ofanalysis:
   a. CFU counts on specificmedium.
   b. Plating for contaminants on specificmedia
   c. Antagonistic capability on target organism bybioassay.
   d. Bioassay procedure based on diseased severity and root colonization as detailed in Appendix-I

Bio-efficacy 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 x 10/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 ¾th 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 x 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 infected. Shoot	Moderately Inefficient (MI)

	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 of 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%.

c. INDIAN STANDARDS ENTOMOPATHOGENIC FUNGI - DRAFT SPECIFICATIONS

1. Form andappearance
2. pH
3. Composition
   a. CFU/g of theproduct
   b. Percent content of the Bio-control organism intheformulation & nature ofbiomass.
   c. Percentage of carrier/filler, wetting/ dispending agent &stabilizers/ emulsifiers, contaminants/ impuritiesetc.
   d. Moisturecontent
4. CFU counts: Minimum $1x10^8$ CFU/ml or gm. (Stability at 30^oC and65%RH).
5. Contaminants:
   a. BiologicalContaminants:
   b. Pathogenic Contaminants: such as gram negative bacteria Salmonella, *Shigella*, *Vibrio* etc:**absent**
   c. Other contaminants should not exceed $1x10^4$/ml org
   d. Chemical/botanical pesticides contaminants: **absent.**
6. Method ofanalysis:
   a. CFU counts by serial dilution and examination under regular compound research microscope with bright fieldoptics.
   b. Plating for contaminants on specificmedia
   c. Entomo-pathogenic capability on target insects bybioassay.

Appendix-I

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

Insect pathogens:

Beauveria bassiana, Metarhizium anisopliae, Nomuraearileyi

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-syntheticdiet)

Stage of insect forbioassay

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 flowsystem

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 discsimilarly.

3. Release ten numbers of second instar larvae of *S. litura* on the leaf surface and incubate the discs in an incubator at 25^0C and 90%RH

4. After 24hours, shift the larvae to the poly-pots containing the semi-synthetic diet and incubate in an incubator at 25^0C and 90%RH

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

6. Lc-50 can be calculated using SPSSpackage

Standard for LC-50: Not more than $2.00X10^6$ spores/ml ($3.0X10^3$ spores/mm^2)

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 $4.00X10^6$ spores/ml (6.0X103 spores/mm^2)

Appendix-II

Bioassay procedure for *Plutellaxylostella*

Various concentrations of *Beauveria bassiana*formulation ranging from 6×10^8 to 2×10^{10} 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 2^{nd}instar larvae of *Plutellaxylostella*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 $25+1^0$C and 70-80% RH for 7 days. Observations on larval mortality are to be made at 3, 5 and 7 days aftertreatment.

Standard for LC_{50} = Not more than 3×10^9 cfu/g

D. INDIAN STANDARDS, ENTOMO-TOXIC BACTERIATECHNICAL /FORMULATION - DRAFT SPECIFICATIONS

S.No.	Details
1.SCOPE	
1.1	This Indian Standard prescribes the requirements and the method of sampling and test for Entomo-toxic bacteria technical and formulation. The product is a bio-pesticide active against target insects. The product is not for human consumption.
2.REQUIREMENTS	
2.1	Common name: i.e., *Bacillus thuringiensis or B. sphaericus etc.*
2.2	Systematic name (Genus, species, serotype, strain and Cry-toxin* along with cry gene)
2.3	Physical specification 2.3.1 Form andappearance 2.3.2 Moisturecontent 2.3.3 pH
2.4	Composition 2.4.1 Delta endo-toxin content (Minimum 2.0%) – estimation as per Appendix-V 2.4.2 Adjuvant 2.4.3 Beta Exo-toxin 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 theorganisms 2.5.2 History (exotic or indigenousstrain) 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	An appropriate test procedure and criteria used for identification, such as morphology, biochemistry and / or serology / immunology 4.1.1 Morphology description, particlesize 4.1.2 Immunology assays: ELISA / Dot blot assay test or any other sensitive standard immunologytest. 4.1.3 Method ofanalysis 4.1.4 Level of beta exo-toxins to be identified if expressed by Housefly bio-assaymethod. 4.1.5 Potency of product by bioassay method(**Appendix-II**) 4.1.5.1 Bio-assaymethod 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) b) Housefly Bioassay method for Beta-exotoxin content (Appendix-IV) c) Determination of toxin content by ELISA / Dot Blot Assay Method(Appendix-V) 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 blotassay).

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 plasticcup.
2. Pour lukewarm diet, approx. 60^0 C, into the cup to a total volume of 30 ml. Place the lid and shake the cup vigorously for a minute to mixproperly.
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 I h to surface sterilize thediet.
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 diluted10-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 thelid.
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 rearingtrays.
7. Keep controls with larvae released on untreated diet, for all theexperiments.
8. The unused rearing trays with diet can be stored in a refrigerator for aweek.
9. Change the diet for the larvae every two or threedays.
10. Record mortality observations at 8 hourly intervals until the end of seven days, for median lethal time LT_{50} calculations. LT_{50} 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 theLT_{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 thirdinstar.

Diet incorporation for filter paper bioassays

1. For bioassays with bollworms, 10 ml toxin incorporated diet is poured over a16 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 alternateday.

2. Record mortality observations until the seventhday.

Surface coating of semi-synthetic diet

1. Prepare the diet and pour it into the trays or the rearing plastic cups. Generally, 10 µl of the toxin can be used to coal 1 sq cm surface area. Gently swirl the diet surface to ensure uniform and complete spread of the solution over the dietsurface.
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 untreateddiet.
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 thebioassay.

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 PDBC, 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)

Exo-toxin determination by PCR studies

Methodology:

Some *Bacillus thuringiensis* strains secrete type I β- exotoxin, which is a non-specific insecticidal and thermo-stable 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 H_2O 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 (In-vitro-gen). The PCR amplification consisted of DNA denaturation at 94°C for 2 min followed by 25 cycles of amplification with a gradient thermo-cycler. Each cycle consisted of a de-naturation 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 betterresults.

Appendix-II

Dot Blot assay of *Bacillus thuringiensis* (Bt) toxin protein as alternate of Bioassay

1) Bt grown till sporulation in shake flask or in fermenter vessel and let the cells lyse and release spore/crystals into themedium

2) Cells are harvested by centrifugation at 10k for 15mins.

3) Wash the pellet with 1M NaCl to remove the B.t. associated seine/metallo proteases and washed twice with sterile distilledwater.

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 15Mins.

5) Supernatant was adjusted to pH 8.0 with Tris HCL pH8.8

6) Protein contents estimated by Lowry'sprotocol.

7) Two fold serial dilutions of test protein were made in PBS and known amount at protein applied on NCP using S&S or Biorad Dot Blot manifold apparatus and applying water vacuum for 30mins.

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 at4^{O}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 withshaking.

11) Remove the NCP from the bag and was several times (as mentioned instep.No.9)

12) Anti-rabbit antibodies conjugated with HRPO/alkaline Phosphate was diluted as per the suppliers instruction and incubated NCP (as in step10)

13) Was as in step11

14) ForHRPO:

    a) Diaminobenzene (4mg/10ml PBS)/4-Chloro-1-Napthol (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. 40N gives bluecolour).
    b) For alkalinePhosphatase:

Alkaline Phosphatase Buffer:

1M TrispH8.8	-10ml/
4MNaCI	- 2.5ml/ make up to100ml
1MMgc12	-0.5ml/

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

15. Stop the reaction by removing the substrate and washing withPBS.
16. Keep on filter paper anddry.

DIFFERENT PROTEIN CONCENTRATION

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

Determination if cell dry weight

Take a known volume of Bacterial culture spin down at 4R formin.

Wash the pellet in minimal distilledwater

Transfer to a pre weighedcontainer

Incubate at 80^0C for 16-18 hours till become dry and weight becomesconstant.

Appendix-III

PURIFICATION OF CRYSTALS BY GELATIN METHOD

Centrifuge the sporulated material and wash pallet 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.

E. INDIAN STANDARDS BACULORIVUS DRAFTSPECIFICATIONS

1.Form and composition of theproduct
1.1 Viral Unit: POB/Capsule count per ml/g of theproduct
1.2 Percent content of the bio-control organism in the formulation and nature ofbiomass
1.3 Percent of carrier/filler, wetting/dispersing agent, stabilizers/ emulsifiers, containments/ impuritiesetc.
2. Moisturecontent
3. pH
4. ViralUnit:

NPVs (*Helicoverpa& Spodoptera*) - $1x10^9$ POB/ml or gm (minimum) (POB –Polyhedral Occlusion Body)

GV (*Chilo, Plutella&Acheae*) - $5x10^9$ Capsules/ml or g. (minimum).

5. **Contaminants**:

5.1 Biologicalcontaminants:

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

5.1.2 **Other microbial contaminants:** Other microbial contaminants should not exceed $1x10^4$ /ml or g

5.2 Chemical/botanical pesticides contaminants should beabsent**.**
6. Identification of Baculovirus by DNA test (Restriction enzyme analysistest).
7. An undertaking should be submitted that the strain is indigenous, naturally occurring and not exotic and not genetically modified as perAnnexure-1.1
8. **Method ofanalysis:**

Viral Unit:

NPVs (*Helicoverpa and Spodoptera*) =$1x10^9$POB/mlor gm.minimum

GVs = $5x10^9$ 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 theformulation:
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 *Chiloinfuscatellus*asdetailedin Appendix-IIIOR

8.2.3 Bioassay for GV against *Plutellaxylostella*as detailed inAppendix-IV.

8.2.4 Bioassay for GV against *Acheaejanta*as detailed inAppendix-V.

8.3 Plating for contaminants on specifiedmedia.

Appendix-I

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

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 chamberfloor.

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 cm^3 =($2.5 \times 10^{-7} cm^3$)

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

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 = 2.5×10^{-7} cm^3

$$\text{Thus, POBcount} = \frac{1000 \times 535}{160 \times 2.5 \times 10^{-7}} = 1.34 \times 10^{10} \text{ POB/ml of testsample}$$

Note: (i) Usually,thisprocedureisrepeated3timesandanaveragetakentogetamore accurateestimate.

(ii)Same procedure will be used for GV also for counting the number ofcapsuleper unit volume of theproduct**.**

Appendix-II

PROCEDURE FOR ESTIMATION OF LC_{50} OF NPV BY THE DIET SURFACE CONTAMINATION METHOD.

i) **Diet to be used**: The standard chickpea-based diet without formalin.
ii) **Bioassay bottles**: 5ml. vials with a diameter of 18 mm (255 mm^2 surfacearea)
iii) **Doses of NPV to betested**:

Helicoverpaarmigera		*Spodoptera litura*	
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 10minutes.

v) **No. oflarvae/dose**:50 (Maintain 50 healthy larvae without virus inoculation forcontrol)

vi) **Stagesof larvae**: II instar larvae (Preferably 4daysold) Release one larva/vial and plug mouth with sterile absorbent cotton. Incubate at 25 ± 1^oC for 7days.

vii) Record mortality in different doses on the 7^{th}day.
viii) Apply Abott's formula for correction of mortality in controltreatment.
ix) Subject the dose – mortality response to probit analysis using relevant statistical software.
x) Express LC_{50} as POB/mm^2 of dietsurface.

Expected standards for NPV for II instar larvae

Species	~~LC 50POB//mm^2~~
1. *Heliocoverpaarmigera*	<0.5
2. *Spodoptera litura*	<20.0

Appendix-III

Bioassay for GV against *Chiloinfuscatellus*

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 x 10^1, 10^2, 10^3, 10^4, 10^5, and 10^6 IBs/larva. One hundred freshlymoulted 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 =< 1x10^3 OB for third instar larvae by micro-feeding.

Appendix-IV

Laboratory bioassay procedures for estimation of LC_{50} of *Plutellaxylostella*(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 beused)
2. PxGV (containing 0.01per cent Triton X 100) of different concentrations 28000, 2800, 280, 28, 2.8 OB/mm^2 on the leaf disc) isprepared
3. Aliquots of 12ul of each concentrating 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 eachtreatment)
4. Air dry these leaf discs for 2-3minutes
5. Repeat the same on the lower surface of the leafdisc.
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 threetimes.
8. Incubate these larvae at25^oC
9. After 24 hours remove the treated leaves (partially eaten) and provide the larvae with fresh cauliflowerleaves.
10. The leaves are changed daily and mortality data recorded everyday.
11. The dosage and time mortality responses are subjected to probitanalysis.
12. If the mortality in the control excess 10% repeat theexperiment.

LC_{50} = < 0.15 OB/mm^2 for second instar larvae by disc method.

Appendix-V

Laboratory bioassay procedures for estimation of LC_{50} 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 5minutes.
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 ($5x10^8$, $5x10^7$, $5x10^6$, $5x10^5$, $5x10^4$ corresponding to 19884, 1988, 198, 19, 1.9 OB per mm^2 on the leafdisc)
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 eachtreatment)
4. Air dry these leaf discs for 5minutes
5. Repeat the same on the lower surface of leafdisc
6. Control leaf discs were treated with distilled water containing 0.02% Tween-80only
7. The leaf discs are placed in Petri dishes (9.0cm dia) line 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 threetimes.
8. Incubate these larvae at25^oC.
9. After 24-48 hours remove the treated leaves (partially eaten) and provide the larvae with fresh castorleaves
10. The leaves are change daily and mortality data recorded everyday
11. The dosage and time mortality responses are subjected to probitanalysis
12. If the mortality in the control exceeds 10% repeat theexperiment.

Recommended LC_{50} GV (*Achaea janata) – LC50* <4 OB/mm^2 for second instar larvae by the leaf discmethod.

ANNEXURE -IV

The Data Requirements for Botanical/ Plant origin Pesticides

II: Plant Based Products

Botanical Pesticides/Pesticides of any Plant Origin like Eucalyptol, Cymbopogon formulation, Neem Based Pesticides, Pyrethrum Extract & Rotenone for Pisci-culture (Formulation), Essential Oils (formulations only) and other similar categories.

General Guidance

1. It is clarified that traditional remedies or products prepared by the individuals for their own-selfuse 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

Sr. No.	Parameters	9(3B)			9(3)				
		TI	TIM	FIM	TI	TIM	FI	FIM	Label expansion
1	Name of the part of the Plant(s) to be used for extraction of the active ingredients	R	R	R	R	R	R	R	-
2	Source of supply of the technial grade material	R	R	R	R	R	R	R	-
3	Outline of Process of formulation	R	R	R	R	R	R	R	-
4	Extract Contents active ingredient	R	R	R	R	R	R	R	-
5	Chemical composition of the formulations	R	R	R	R	R	R	R	-
6	Physico-chemical properties of a.i.& adjuvants	R	R	R	R	R	R	R	-
7	Product Specifications as BIS format	R	R	R	R	R	R	R	-
8	Method of analysis (a.i.)	R	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	R	-
10	Analytical test report (generated by the NABL / GLP certified lab	R	R	R	R	R	R	R	-

	from the source of manufacturing country)								
11	Shelf-life claim (In the case of)	R	R	R	R	R	R	R	-
12	Shelf-life data **(# in the case of Eucalyptus based products containing 'Eucalyptol', Rotenone for pisci-culture, Cymbopogan spp.)**	R/# NR	R/#NR	R/#NR	R	R	R	R	-
13	Process of mfg in step wise manner along with the list of RAW material with impurity	R	R	R	R	R	R	R	-
14	Submission of sample to CIL along with slandered	R	R	R	R	R	R	R	-
15	Drawl of in process of sample to submission of CIL.	NR	R	NR	NR	R	NR	NR	-
16	Copy of RTT permit	R	NR	NR	R	NR	R	NR	-
17	DNA from the source country along with copy of Registration (CR) issued from importing country	R	NR	NR	R	NR	R	NR	-
18	In case of import consent letter from source of supply	R	NR	NR	R	NR	R	NR	-

Bio-efficacy

Sr. No.	**Parameters**	**9(3b)**			**9(3)**					**9(4)**
		TI	**TIM**	**FIM**	**TI**	**TIM**	**FI**	**FIM**	**Label expansion**	**TI/TIM/FI/FIM or endorsement of already approved label expansion**

1	Bio-effectiveness against target pest and disease in specified crops	R	R	R*	R	R	R	R**	R**	No data requirement
2	Phytotoxicity (as per standard tests)	R	R	R*	R	R	R	R**	R**	
3	Compatibility with other agro-chemicals, if claimed	NR	NR	R	NR	NR	R	R	R	
4	Stability of formulation (Photo, thermal etc.) in aqueous dilution (acidic, neutral & basic)	NR	NR	R	NR	NR	R	R	R	
5	Direction concerning dosage for each target pest and disease	NR	NR	R	NR	NR	R	R	R	
6	Stage of crop for use and stage of target pest and disease	NR	NR	R	NR	NR	R	R	R	
7	Waiting period	NR	NR	NR	NR	NR	NR	NR	NR	
8	Application equipment	NR	NR	R	NR	NR	R	R	R	
9	Information regarding registration status in other countries, if any.	NR	NR	R	R	NR	R	R	R	
10	Time of Application	NR	NR	R	NR	NR	R	R	R	
11	Purpose of Manufacture	R	R	R	R	R	R	R	R	
12	Residue in plant	NR	NR	NR	NR	NR	NR	NR	NR	
13	Residue in soil	NR	NR	NR	NR	NR	NR	NR	NR	
14	Registration status in foreign countries	NR	NR	R	R	NR	R	R	R	
15	Residue tolerance limit fixed by foreign countries	NR	NR	NR	NR	NR	NR	NR	NR	

R[*]: Two seasons /year data generated at minimum two different agro climatic zones
R[**]**:** Two seasons / year data generated at minimum three different agro-climatic zones
NR: Not required

Toxicology

Sr. No.	Parameters	9 (3b)			9(3)					9(4)
		TI	TIM	FIM	TI	TIM	FI	FIM	Label expansion	TI/TIM/FI/FIM or endorsement of already approved label expansion
1	Acute Oral Toxicity-Rat	R	R	R	R	R	R	R	-	NR
2	Acute dermal Toxicity-Rabbit	R	R	R	R	R	R	R	-	NR
3	Acute Inhalation-rat	R	R	R	R	R	R	R	-	NR
4	Primary Skin Irritation-Rabbit	R	R	R	R	R	R	R	-	NR
5	Primary Eye Irritation-Rabbit	R	R	R	R	R	R	R	-	NR
6	Skin sensitization-Guinea pig	R	R	R	R	R	R	R	-	NR
7	Sub-acute oral-rat	NR	NR	NR	NR/R*	NR/R*	NR	NR	-	NR
8	Sub-acute oral-dog**	NR	NR	NR	R	R	NR	NR	-	NR
9	Sub-acute dermal	NR	NR	NR	R	R	NR	NR	-	NR
10	Sub-acute inhalation	NR	NR	NR	R	R	NR	NR	-	NR
11	Neuro-behavioral toxicity	NR	NR	NR	R	R	NR	NR	-	NR
12	Teratogenicity	NR	NR	NR	R	R	NR	NR	-	NR
13	Effect on Reproduction	NR	NR	NR	R	R	NR	NR	-	NR
14	Carcinogenicity/chronic	NR	NR	NR	R	R	NR	NR	-	NR

	toxicity/combined toxicity study									
15	Metabolism	NR	NR	NR	R	R	NR	NR	-	NR
16	Mutagenicity* (AMES+2in-vitro+1 in-vivo)	NR	NR	NR	R	R	NR	NR	-	NR
17	Toxicity to birds	R (Two species)	R (Two species)	R (one species)	R (Two species)	R (Two species)	R(one species)	R (one species)	-	NR
18	Toxicity to fresh water fish	R	R	R	R	R	R	R	-	NR
19	Toxicity to honey bees	R	R	R	R	R	R	R	-	NR
20	Toxicity to earthworm	R	R	R	R	R	R	R	-	NR
21	Medical data	R	R	R	R	R	R	R	-	NR
22	Human toxicity information	NR/R*	NR/R*	NR/R*	NR/R*	NR/R*	NR/R*	NR/R*	-	NR
23	Health record of industrial workers	NR	NR	R	NR	NR	R	R	-	NR
24	International report on carcinogenicity & genotoxicity study	NR/R	NR/R	NR/R	NR/R	NR/R	NR/R	NR/R	-	NR

Note:

1. The requirement for sub-acute studies shall be determined on the basis of results of other toxic study reports.
2. * Please refer to guidance document on toxicology for registration of chemical pesticides in India

Toxicity Study No. 8: Sub-acute oral toxicity study (Dog): ** Peer reviewed international published literature is also required for dog study.

Note: 1) The general recommendations as mentioned in the guidance document on toxicology for registration of chemical pesticides in India will also be applicable for registration of botanical pesticides on merit case to case basis

2) Waiver would be considered only when existing information provides robust and full scientifically sound weight of evidence approach and read across / bridging from structurally and / or biologically related similar pesticides specifically case to case basis on full merits.

3) The replacement alternatives not involving experiments on animals would be considered in those cases where in case of alternatives if available with full and sound justification is provided specifically case to case basis on merit.

Packaging

Sr. No.	Parameters	9 (3b)			9(3)					9(4)
		TI	TIM	FIM	TI	TIM	FI	FIM	Label expansion	TI/TIM/FI/FIM or endorsement of already approved Packaging
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	N.A	R*
2	Manner of labeling and leaflet	R	R	R	R	R	R	R	N.A	R*
3	Type of packaging (Ultra small, small or Big whichever is applicable	R	R	R	R	R	R	R	N.A	R*
4	Manner of packaging	R	R	R	R	R	R	R	N.A	R*
5	Specification for primary, secondary and transport packages (whichever is applicable)	R	R	R	R	R	R	R	N.A	R*
6	Details of packaging material and its	R	R	R	R	R	R	R	N.A	R*

	compatibility with content									
7	Performance of container with content during storage stability test (Shelf life study)	R**	R	R	R	R	R	R	N.A	R*
8	Transport worthiness test	R**	R	R	R	R	R	R	N.A	R*

Note: R*- Required only if manner of packaging is different then the 9(3) registrant.
R**- Before commercialization the data will be required.
N.A-Not Applicable

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)and manner of packaging 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 in consonance of BIS or product specification.
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^{o}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

Definitions as per OECD:

Semio-chemicals are chemicals emitted by plants, animals, and other organisms, and synthetic analogues of such substances, that evoke a behavioural or physiological response in individuals of the same or other species. They include pheromones and allele-chemicals. Here, Semio-chemicals refers to only those that affect the behavior of arthropods.

Pheromones are Semio-chemicals produced by individuals of a species that modify the behavior of other individuals of the same species (i.e. an intra-specific effect).

Straight-chained lepidopteran pheromones (SCLPs) are a group of pheromones consisting of un-branched aliphatic having a chain of nine to eighteen carbons, containing up to three double bonds, ending in an alcohol, acetate or aldehyde functional group.

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 (If impurities in SCLP are also SCLPs, they should be summed-up)	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 50%	R	R	

	attractantcy 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 *Helicovarpa armigera*,

Spodoptera litura, *Earisa spp*. etc.

Colour: Any colour other than black.

Structure: The funnel trap may have three parts (1) canopy (2) funnel shapped "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 dislodged 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 M1 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**	**Semio-chemicals / pheromones**		
		9(3b)	**9(3)**	**9(4)**
	Acute oral toxicity- rat	R	R	No data required if chemical equivalence is established with 9(3) registrant
	Acute dermal toxicity- rat	R	R	
	Acute Inhalation – rat	R	R	
	Primary Skin Irritation - Rabbit	R	R	
	Primary Eye Irritation - Rabbit	R	R	
	Skin Sensitization - Guinea pig	R	R	
	Sub- acute oral- rat	NR	NR/R	
	Sub-acute dermal	NR	NR/R	
	Sub- acute inhalation	NR	NR/R	
	Neurotoxicity	NR	NR/R	
	Teratogenicity	R	NR/R	
	Effect on Reproduction	NR	NR/R	
	Carcinogenicity/chronic toxicity/combined toxicity study*	NR	NR/R	
	Metabolism	NR	NR/R	
	Mutagenicity (AMES + 2 in-vitro +1 in-vivo)*	R	NR/R	
	Cellular immune response	R	R	
	Toxicity to bird (2species)*	R	NR/R	

	Toxicity to fresh water fish	R	NR/R	
	Toxicity to Honey bees	NR	NR/R	

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 toxicitydata are required unless it is added with some other pesticides.
3. Data for 9(3) will be conditionally required on case to case basis.

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

Note: 1) The general recommendations as mentioned in the Guidance document on Toxicology for registration of chemical pesticides in India will also be applicable for registration of Semio-chemicals / Pheromones on merit case to case basis.

2) Waiver would be considered only when existing information provides robust and full scientifically sound weight of evidence approach and read across/bridging from structurally and /or biologically related similar pesticides specifically case to case basis on full merits.

3) The replacement alternatives not involving experiments on animals would only be considered in exceptional cases, in case of alternatives if available with full and sound justification is provided specifically case to case basis on merits subject to the full satisfaction of the expert regarding toxicity data provided for the alternatives.

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	Semio-chemicals (pheromones)		
		9(3b)	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^{o}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 semio-chemical 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. **Semio-chemicals:** Chemicals emitted by a plant or animal that evoke a behavioral or physiological response in another organism. When the semio-chemical 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.

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