

# Manual for conduct of multilocational trials of transgenic silkworm hybrids under contained facilities



Prepared by

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&

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## **SECTION-1: INTRODUCTION TO THE PROJECT AND REGULATORY REQUIREMENTS**

### **1.1 Background**

The Centre for DNA Fingerprinting and Diagnostics (CDFD), Hyderabad has developed transgenic silkworm strains resistant to Nuclear Polyhedrosis Virus (NPV) infection using RNAi technology under the Centre of Excellence on Genetics and Genomics of silkmoths. The antiviral property of the baculoviral resistant transgenics in the Nistari genetic background was transferred to a high yielding, baculovirus susceptible bivoltine commercial silkworm strain, CSR2 through transgene (dsRed marker phenotype) selection coupled with microsatellite marker-assisted screening and repeated backcrossing. When these transgenic lines were infected with baculovirus, they provided >80% resistance compared to non-transgenic control lines. A brief note about the technology is placed at **Annex-1**. CDFD has collaborated with Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur and Central Silk Board (CSB), Bangalore for taking forward this technology.

Nistari and CSR2 transgenic lines were developed during Phase I of the project “Centre of Excellence on Genetics and Genomics of Silkmoths (CoE-I)”, supported by the Department of Biotechnology, Ministry of Science & Technology, Government of India. These lines were then transferred to user dependants: APSSRDI and CSB for maintenance. During phase II of CoE-I, transgenic hybrids were generated by crossing Nistari and CSR2 transgenic silkworm lines with various commercial breeds. The performance of the hybrids was tested against baculoviral (BmNPV) infection and the data on their survival (pupation %) was recorded. The best performing hybrids were selected for multilocational trials. The work on the transgenic silkworm was published in the journal Genetics 193: 63-75 in 2013 and a patent was filed.

Since transgenics are regulated under Rules for the manufacture, use, import, export & storage of hazardous micro organisms/ genetically engineered organisms or cells, 1989, under the Environment (Protection) Act, 1986, there is a need to conduct a series of multilocational trials and biosafety studies for commercialization of the technology. Accordingly, the CDFD prepared a roadmap for conducting field trials and generating data for biosafety assessment with support from Biotech Consortium India Limited (BCIL). CDFD then approached the Review Committee on Genetic Manipulation (RCGM) for seeking biosafety regulatory approvals to carry out multilocational contained trials of the transgenic silkworms. After a series of presentations and clarifications, RCGM has conveyed its approval for conduct of multilocational trials in contained facilities in two phases (initially in institutions/nested units followed by at farmer’s levels), subject to constitution of IBSCs in each participating centre and constitution of a Co-ordinating Committee. Accordingly, all the participating institutes namely, Andhra Pradesh State Sericulture Research and Development Institute (APSSRDI), Hindupur, Central Sericultural Research and Training Institute (CSRTI), Mysore, CSRTI, Pampore and CSRTI, Berhampore have their IBSCs in place

and recommended the conduct of trials at their premises/nested units. As suggested by RCGM, a Coordinating Committee has also been constituted under the chairmanship of Director, APSSRDI with members from all participating institutes for effective monitoring of the contained trials. The permit letter has since been received from RCGM for initiating the trials (**Annex-2**). The funding support to initiate these trials has been received from Biotechnology Industry Research Assistance Council (BIRAC), to CDFD. The grant in aid include support for CDFD, APSSRDI and Central Silk Board (participating institutions).

This manual has been prepared to familiarize all participating centres with the regulatory requirements to be followed during the conduct of the trials.

## **1.2 Objectives of the project**

The objective to conduct multilocational trials in contained facilities on GE BmNPV resistant *Bombyx mori* to establish their efficacy and generate data on parameters determining the resistance of transgenic lines against NPV for further steps in regulatory process. It is proposed to fulfil the following objectives:

- i. Conduct of Phase 1 multilocation trials at Institutions level to establish efficacy of the GE BmNPV resistant *Bombyx mori*;
- ii. Conduct of repeated multilocation trials at farmer's facilities to collect the relevant data for regulatory approvals; and
- iii. Regulatory approval for release of transgenic silkworms

As on date, Phase I trials have been permitted by RCGM.

## **1.3 Rearing facility**

A standard rearing house is to be used for conduct of contained trials. Although, the *Bombyx mori* cannot move and disseminate on its own, all precautions are to be taken to ensure that no accidental escape takes place. The eggs of F1 hybrids will be transported from APSSRDI to trial sites in standard egg cases/ transport boxes. All controls are to be reared in similar conditions so that the performance of transgenic lines will be compared with respective controls. All equipment to be used during the rearing process, is to be cleaned as per standard procedures. The transgenic silkworm bed refuse has to be shifted to separate pit secured with a mesh in order to prevent from predators, if any.

Proper monitoring is to be ensured at all stages to prevent any transfer of GE material by human intervention or the entry of predators like uzi fly.

## **1.4 Regulatory compliance during conduct of trials**

As per the conditions stipulated in the permit letter by RCGM, all Trial In-charges are required to follow the standard operating procedures (SOPs) during the conduct of the trials. Accordingly, a set of SOPs specific for

*Bombyx mori* and accompanying recording formats for compliance have been prepared and placed at section 2 and 3 of this document respectively.

#### **1.4.1 Standard Operating Procedures (SOPs)**

- i. Transport of regulated GE silkworm material
- ii. Storage of regulated GE silkworm material
- iii. Management of trials in contained facilities
- iv. Management of harvest and termination of trials

#### **1.4.2 Compliance recording formats (CRF)**

- i. Record of Transport & Transport Inventory List
- ii. Record of Storage
- iii. Record of Storage Inspection & Inventory
- iv. Record of Brushing
- v. Record of Harvest/Termination
- vi. Record of Corrective Action

#### **1.4.3 Display Board**

A display board is to be placed outside each rearing facility and the following items are to be included on the display board:

- i. Trial-in-charge's name and contact details
- ii. Permit number from the regulatory authority
- iii. Trial initiation date
- iv. Duration of the trial
- v. Hybrids under evaluation
- vi. No. of rearings and no. of cycles.

#### **1.4.4 Visitors register**

As indicated in the permit letter, only authorized personnel are to be allowed to visit the experimental facility and persons visiting the experimental facility have to enter the name, designation and purpose of the visiting the experimental facility in a bound book which should be made available to the regulatory authorities when requested for.

In line with the above, the Trial-in-charges are required to maintain a bound book and keep all the records of visitors.

#### **1.4.5 Photographs of trials**

Minimum of 3-4 photographs of experimental facility are required to be taken from a distance sufficient to indicate the experiment in a single photograph. The suggested intervals during the trial for taking the photographs are at the start of the experiments and different stages from brushing to spinning (3<sup>rd</sup> instar, 5<sup>th</sup> instar and spinning stage). These photographs shall make part of the trial report.

- 1.5** Minimum of 3-4 photographs of experimental facility are required to be taken from a distance sufficient to indicate the experiment in a single photograph. The suggested intervals during the trial for taking the photographs are at the start of the experiments and different stages from brushing to spinning (3<sup>rd</sup> instar, 5<sup>th</sup> instar and spinning stage). These photographs shall make part of the trial report. **DATA TO BE RECORDED**

During the conduct of each trial, data is to be recorded on parameters for comparing the efficiency and any other changes in the genetically engineered BmNPV resistant *Bombyx mori* vs. controls. The data recording formats (DRF) are placed in section 5 and are to be completed for each trial.

- i. Brushing report
- ii. Log sheet (daily activity chart)
- iii. Index card
- iv. Rearing performance of transgenic hybrids
- v. Reeling performance of transgenic hybrids
- vi. Quality characteristics of raw silk reeled

## **1.6 Visits of the monitoring team**

The trials shall be monitored as follows:

- i. Central Compliance Committees (CCCs), as constituted by RCGM
- ii. Internal monitoring through expert team by the Chairman of the Coordination Committee

As these inspection or monitoring can occur at any point during the conduct of the trial, it is important to ensure that all the data recording (compliance as well as the data) is maintained up-to-date, in accordance with conditions stipulated by RCGM and the SOPs.

## **1.7 Trial report**

The original record of the trial data must be retained by the Trial-in-charge during the conduct of the trial and forwarded to the Chairman, Coordination Committee after completion of the trials. A trial report shall be prepared by the Permitted Party summarizing the completed trials including methods, observations and data collected and submitted to RCGM.

## **SECTION-2: STANDARD OPERATING PROCEDURES (SOPs) FOR TRIALS OF REGULATED, GENETICALLY ENGINEERED (GE) SILKWORM (*Bombyx mori*) IN CONTAINED FACILITIES**

### **1. INTRODUCTION**

Standard Operating Procedures (SOPs) have been prepared to provide guidance for the conduct of the trials of regulated, genetically engineered (GE) silkworm (*Bombyx mori*) in contained facilities in India. The regulated GE silkworm seed/ eggs or live silkworm material are transported to the trial site by airlifting/ courier service by road to reach the designated place well in advance. The acid treated silkworm eggs are cold stored as per the recommendations to obtain good hatching. Management of contained trials of the designated GE silkworm will be carried out as per the programme. This includes scientific method of incubation, young age silkworm (chawki) rearing, satisfactory disinfection of rearing facility and maintenance of quality mulberry leaf followed by shoot rearing. Post-trial activities involve assessment of cocoon quality and reeling data (renditta, reelability and quality of raw silk). Therefore, in view of the above, SOPs have been prepared for the following steps involved during conduct of the trial:

- v. Transport of regulated GE silkworm material
- vi. Storage of regulated GE silkworm material
- vii. Management of trials in contained facilities
- viii. Management of harvest and termination

### **2. GENERAL REQUIREMENTS**

2.1 The Permitted Party and all other agents acting on behalf of the Permitted Party must comply with these SOPs.

2.2 No regulated GE silkworm material from the trial site is permitted to enter any commercial activities related to silk production.

2.3 All the relevant Records are to be filled as per the requirements indicated in each SOP. The following formats of records have been enclosed:

- vii. Record of Transport & Transport Inventory List
- viii. Record of Storage
- ix. Record of Storage Inspection & Inventory
- x. Record of Brushing
- xi. Record of Harvest/Termination
- xii. Record of Corrective Action

2.4 In situations where it becomes known that there has been noncompliance with the terms and conditions of the contained trial permit due to any reasons, the Permitted Party must inform RCGM/GEAC immediately by telephone and positively within 24 hours in writing. RCGM/GEAC will provide the Permitted Party with the appropriate course of remedial action. The corrective actions should be appropriately recorded as explained in the subsequent sections.

### 3. TERMINOLOGY

The following terminology has been used in the SOPs:

- i. **Accidental release:** Any unintended release of regulated silkworm material into the commercial activities related to silk production.
- ii. **Facility In-Charge:** For the purpose of this SOP, shall be the person designated by the Permitted Party as responsible for the storage of the regulated material.
- iii. **Contained trial:** The rearing of one or more regulated events in a single experimental station.
- iv. **GEAC:** Genetic Engineering Appraisal Committee.
- v. **Genetic engineering:** The technique by which heritable material, which does not usually occur or will not occur naturally in the organism or cell concerned, generated outside the organism or the cell is inserted into said cell or organism. It shall also mean the formation of new combinations of genetic material by incorporation of a cell into a host cell, where they occur naturally (self-cloning) as well as modification of an organism or in a cell by deletion and removal of parts of the heritable material (*Rules, 1989*).
- vi. **Containment (adopted from rDNA safety guidelines, 1990):** The term “containment” is used to describe safe methods for managing infectious agents and/or regulated GMOs/LMOs/rDNA material in the laboratory environment where they are being handled or maintained.
- vii. **Packaging Material:** The material used to secure regulated, genetically engineered silkworm material for the purpose of transport and storage. Examples include egg transportation boxes, envelope, cardboard box, nylon nets, polythene bags.
- viii. **Permitted Party:** The sponsoring organization identified on contained trial permit issued by RCGM or GEAC who shall accept full responsibility for compliance with all terms and conditions of the permit.
- ix. **Silkworm material:** Various stages of life cycle of *Bombyx mori*. such as eggs, larvae, pupa, moth, cocoons, silk skeins.
- x. **Primary container:** The container into which regulated GE silkworm material is placed (e.g. egg transportation boxes, envelope, cardboard box, nylon nets, polythene bags).
- xi. **Recipient:** For the purpose of this SOP, shall be the Permitted Party, Trial In-Charge or Facility In- Charge.
- xii. **RCGM:** Review Committee on Genetic Manipulation.
- xiii. **Regulated GE Silkworm:** Any silkworm produced through genetic engineering, including eggs or live material derived from that species, which has not been authorized by the Government of India for commercial release pursuant to *Rules, 1989*.
- xiv. **Rules, 1989:** Rules for the Manufacture, Use, Import, Export and Storage of Hazardous Micro-Organisms, Genetically Engineered Organisms or Cells Rules, 1989 conferred by sections 6, 8 and 25 of the Environmental (Protection) Action, 1986.
- xv. **Secondary container:** The container into which a primary container is placed.
- xvi. **Transport In-Charge:** The person identified by the Permitted Party as being responsible for the transport of regulated GE silkworm material.

xvii. **Trial In-Charge:** The person/scientist designated by the Permitted Party as responsible for ensuring compliance with the terms and conditions of a contained trial permit and providing information required by regulatory bodies.

xviii. **Trial site:** A single site where one or more trials of the same silkworm species are conducted in contained facilities

xix. **Trial site location:** The geographic location of a contained trial site *e.g.*, address

#### **4. STANDARD OPERATING PROCEDURES**

The following SOPs should be followed for conducting trials of regulated, GE silkworm (*Bombyx mori*) in contained facilities in India.

##### **A. STANDARD OPERATING PROCEDURE (SOP) FOR THE TRANSPORT OF REGULATED GE SILKWORM MATERIAL**

###### **A.1 Scope**

A.1.1 This SOP applies to the transport of regulated, GE silkworm eggs/ seeds or live silkworm material for the purpose of import, export, inter-state movement and intra-state movement.

###### **A.2 General Requirements**

A.2.1 All regulated, GE silkworm eggs/seed or live silkworm material must be stored in secure containers or, egg transportation boxes as per the requirement.

A.2.2 All regulated, GE silkworm material must be kept in a separate (secured in a primary container) from other non-GE silkworm material during transport.

A.2.3 All regulated GE silkworm material must be clearly labelled i.e. number, quantity, date of preparation (treated/ hibernated, date of release and probable date of hatching as in case of silkworm eggs).

A.2.4 The supply agency will ensure that appropriate containers/ packaging materials are supplied to all agents working on their behalf for the purpose of transporting regulated, GE silkworm material.

###### **A.3 Specific Requirements for the Transport of Regulated GE Silkworm eggs or live silkworm material**

A.3.1 The requirement of this section will also apply for the non-regulated (control) silkworm eggs that will accompany with regulated, GE silkworm eggs for testing when transported within the same secondary container.

A.3.2 Regulated GE silkworm eggs will be secured within a primary container (egg transportation box). The primary container carrying eggs will be within a



sealable cotton bags/ polythene constructed of tear and moisture resistant material.

A.3.3 More number of primary containers (loose egg transportation boxes) will be placed within a sealable, leak-proof single secondary container.

A.3.4 The secondary container should be resistant to breakage and dampness consisting of corrugated fibreboard, corrugated cardboard or wood.

A.3.5 The primary (loose egg transportation box, envelope, cardboard box, polythene bags, nylon nets) and secondary containers (cotton bag or cardboard box) used to transport regulated, GE silkworm material are invariably destroyed (by autoclaving or burning) after use.

A.3.6 Any residual silkworm material (i.e. unhatched eggs/ dead larvae/ pupa/ moth/ cocoon) recovered during the process of cleaning must be rendered non-viable by heating, incineration or crushing.

A.3.7 Primary and secondary containers should be labelled in accordance with the requirements of Section A.4.

A.3.8 Prior to sending the material, the Transport In Charge must inform the Recipient of dispatch of the material as outlined in A.5.

#### **A.4 Labelling of Containers**

A.4.1 Primary containers should be labelled with an identifying code or name of the hybrid and the Dispatch Number found on the Record of Transport.

A.4.2 All secondary containers used to transport regulated GE silkworm material should be labelled to identify the Transport In Charge and Receiver and their emergency contact details (i.e. Telephone No, Mobile No, Email etc.) in case of an accidental release.

#### **A.5 Accompanying Documentation for the Transport of Regulated GE Silkworm eggs or live silkworm material**

A.5.1 The supply agencies (in-charge) must complete the following sections of the Record of Transport: contact details of supplier and recipient, regulated GE Silkworm material identification code; pre-transport details should include his/her signature; and date of dispatch.

A.5.2 When multiple primary containers are included within a single secondary container, a Transport Inventory List must be attached to the Record of Transport. The list should include number of egg boxes, probable date of hatching & release of eggs, designated hybrids, destination, consignment number etc).

A.5.3 The details of Record of Transport, with attached Transport Inventory List if applicable, should be intimated in writing (email/fax/letter) to the Receiver before the consignment is sent.

A.5.4 The original Record of Transport, with attached Transport Inventory List if applicable, must be placed within the secondary container (cardboard box) by the supplier/ Transport In-Charge.

A.5.5 Copies of the Record of Transport and Transport Inventory List if applicable and any other accompanying documents must be retained by the Transport In-Charge.

## **A.6 Receipt of Transported GE silkworm eggs or live silkworm material**

A.6.1 When a consignment of regulated, GE silkworm eggs or live silkworm material is received; the following actions should be undertaken immediately by the recipient:

- i. Confirmation/Verification of the consignment and the Record of Transport and Transport Inventory List (if applicable) accompanying the consignment.
- ii. If the Record of Transport is absent from the consignment, the recipient must contact the supplier and request that a copy be sent/transmitted immediately.
- iii. Until such time as the Record of Transport is received, the consignment must be placed in storage and no further action shall be taken. On receipt of Record of Transport, the rest of this SOP shall be followed.

A.6.2 The Recipient shall complete the details regarding Receipt of Consignment section of the original Record of Transport.

A.6.3 If the secondary container was damaged during transport, the Recipient must ensure that the primary container (egg transportation box/ boxes, envelope, polythene bags, nylon nets) were not damaged and that none of the regulated GE silkworm material was lost by confirming the number (in case of sheet eggs) or weight (in case of loose eggs or cocoons) of the consignment as the case maybe.

A.6.4 If it is suspected that an accidental release has occurred, the corrective action requirements provided at the end of the SOP must be followed.

A.6.5 A copy of the completed Record of Transport should be sent in writing (either email or fax) by the Recipient to the supplier (Transport In-Charge).

## **B. STANDARD OPERATING PROCEDURE (SOP) FOR THE STORAGE OF REGULATED GENETICALLY ENGINEERED SILKWORM MATERIAL**

### **B.1 Scope**

B.1.1 This SOP applies to the storage of regulated, GE silkworm material in India.

## **B.2 Specific Requirements for the Storage of Regulated silkworm material**

B.2.1 The Permitted Party/Facility In-Charge must ensure the suitability of all storage facilities prior to accepting consignments of regulated silkworm material.

B.2.2 A storage area must be a fully enclosed space and must be secured by a lockable door. Any windows (if present), must be closed and locked.

B.2.3 The storage area used to store the samples of regulated silkworm material (from both control and hybrid), each sample should be stored separately in a sealed, labelled container in accordance with the requirements of Section B.3 of this SOP.

B.2.4 Access to storage areas must be limited to personnel authorized by the Permitted Party and shall accept responsibility for compliance with all terms and conditions of the permit.

B.2.5 Areas or units designated for storage of regulated silkworm material must be cleaned immediately following the period of storage.

B.2.6 The addition of regulated silkworm material to the storage area or removal of the material must be recorded on the Record of Storage Inspection and Inventory at an appropriate time.

B.2.7 Over consigned GE silkworm material removed from storage for the purpose of disposal must be rendered non-viable by heating or burning.

## **B.3 Labelling of the Storage Area**

B.3.1 The storage area must be labelled as containing regulated GE silkworm material (see Section B.7 for a sample label).

B.3.2 The storage area label should be affixed to the point of entry to the storage area.

## **B.4 Inspection of the Storage Area**

B.4.1 Inspection of the storage area must be completed one every two weeks by the Identified and Designated Party. Facility-in-charge must ensure that the storage conditions (temperature and humidity) are maintained in accordance with the Standard Operational Procedures. Each inspection is to be recorded on the Record of Storage Inspection and Inventory.

B.4.2 The Record of Storage Inspection and Inventory is to be retained by the Permitted Party/Designated Party/ Facility In-Charge.

## **B.5 Inspection by Regulatory Officials**

B.5.1 Access to the storage facility for the purpose of inspection will be provided to regulatory officials/ monitoring committees upon request for official purposes preferably during regular working hours.

## **B.6 Occurrence of Non-Compliance**

B.6.1 In situations where non-compliance with the terms and conditions of the contained trial permit is confirmed, the Permitted Party will notify RCGM/GEAC immediately by telephone and positively within 24 hours in writing. RCGM/GEAC will provide the Permitted Party with the appropriate course of remedial action.

## **B.7 Sample Storage Area Label**

**THIS STORAGE AREA CONTAINS REGULATED GENETICALLY  
ENGINEERED SILKWORM MATERIAL**

Storage Site Address

Room Number or Description:

**Access to this storage area is limited to Personnel designated  
by the Permitted Party:**

Name of facility in-charge

Room no.

Telephone no.

**In case of emergency or damage to the storage area, contact  
the facility in-charge immediately**

## **C. STANDARD OPERATING PROCEDURE (SOP) FOR THE MANAGEMENT OF CONTAINED TRIALS OF REGULATED GE SILKWORM**

### **C.1 Scope**

C.1.1 This SOP applies to all contained trials of regulated, GE silkworm in India.

### **C.2 Requirements for brushing and rearing of GE Silkworm in contained facilities**

C.2.1 All equipment and tools used in the maintenance of the rearing house must be cleaned on the trial site prior to their removal to eliminate unintended transport of regulated GE silkworm material from the trial site. The general procedure (accepted) of cleaning includes washing of the rearing house and

equipment's with bleaching powder followed by disinfection (bleaching powder). Any regulated silkworm material (such as dead cocoon, floss, moths etc.) recovered must be rendered non-viable by burning or burial at the trial site.

C.2.2 An action plan of the trial/ Trial Protocol must be prepared by the Trial-in-charge. Instructions for the preparation of trial are to be provided to the test centres.

C.2.3 A systematic record of brushing must be completed for each trial. A copy of the Record of brushing, along with the appended rearing action plan/ Trial Protocol, must be submitted to RCGM/GEAC within seven (7) days following the start of trial. The original data recording format for rearing performance of transgenic hybrids must be retained by the Trial In-Charge, and copies made available to regulatory officials upon request.

C.2.4 The Trial In-Charge must place a circular in the notice board at the trial (rearing) site indicating the purpose and duration of the contained trials and the authorization under which the contained trials were approved.

C.2.5 The Trial In-Charge must ensure that only designated personnel authorized by the agency (Permitted Party) are allowed on the trial of rearing. A note book (visitor's book) including the name, address and affiliation must be maintained of all personnel who enter the rearing house.

### **C.3 Performance Requirements for Contained Trials**

C.3.1 Any regulated silkworm material removed during maintenance of the trial must be rendered non-viable by burning or burial at the trial site.

C.3.2 The rearing facility must be contained through the trial period and the Trial In-Charge must ensure that the trial facility are checked for by implementing a program of regular monitoring.

### **C.4 Monitoring of the Contained Trial by the Trial In-Charge**

C.4.1 The designated Trial In-Charge must monitor the trial (rearing) site at least once in each instar from time of brushing and during spinning until the time of harvest of the cocoons/ trial.

### **C.5 Inspection by regulatory officials**

C.5.1 Access to the trial (rearing) site for the purpose of inspection will be provided to regulatory (designated) officials/monitoring committees upon request, for official use only and preferably during regular working hours.

### **C.6 Record Keeping**

C.6.1 Data recording format for rearing performance of transgenic hybrids and photographs of each trial facility will be retained by the Trial In-Charge.

C.6.2 Original copy (appropriate) of the record of brushing

C.6.3 The records associated with the management of contained trials must be available for inspection by RCGM/GEAC, State Government Officials or their nominee upon request.

C.6.4 The original copies of all the reports related to conduct of the trial are to be forwarded to the Permitted Party by the Trial-in-charge, when all the trials have been completed.

C.6.5 The records of all permitted contained trials for a minimum of five (5) years, whether or not the regulated GE Silkworm hybrid is authorized for commercial release, have to be made available (archive copies) by the permitted party.

C.6.6 Record of corrective action (if applicable)

## **D. STANDARD OPERATING PROCEDURE (SOP) FOR THE HARVEST OR TERMINATION OF CONTAINED TRIALS OF REGULATED GE SIKWORM**

### **D.1 Scope**

D.1.1 This SOP applies to the harvest or termination of contained trials of regulated, GE Silkworm

### **D.2 Requirements for Harvest/ Termination of Contained Trials**

D.2.1 The requirements in this section apply to the harvest or termination of contained trials

D.2.2 All equipment and tools used during harvest or termination of contained trials must be cleaned on the trial site prior to their removal to eliminate unintended transport of regulated GE silkworm material from the trial site. Acceptable methods of cleaning include disinfection by 5% bleach or 2.5% formalin. Any silkworm material recovered must be rendered non-viable by burning or burial on the trial site.

D.2.3 A Record of Harvest/Termination will be completed for each trial site. This Record will document the amount and fate of all harvested material (cocoon) and the disposal of any unwanted silkworm material on the trial site. The Record of Harvest/Termination must be retained by the Trial In-Charge, and copies made available to regulatory officials/monitoring committees upon request.

### **D.3 Destruction of Regulated GE Silkworm Material**

D.3.1 Silkworm material (i.e, dead cocoons, floss, moths etc) from trial site that is not retained for the purpose of silk production will be destroyed by burning or burial on the trial site.

D.3.2 The Trial In-Charge must monitor harvest or termination at trial sites to ensure that all regulated silkworm material that is not retained is disposed of as described above.

#### **D.4 Transport of Harvested Materials from the Trial Site**

D.4.1 The transport of cocoons from the trial site will be conducted in accordance with the Standard Operating Procedure for the Transport of Regulated GE Silkworm eggs.

#### **D.5 Inspection By Regulatory Officials**

D.5.1 Access to the trial site for the purpose of inspection will be provided to regulatory officials/monitoring committees upon request for official purposes preferably during regular working hours.

#### **D.6 Occurrence of Non-Compliance**

D.6.1 In situations where non-compliance with the terms and conditions of the contained trial permit is confirmed, the Permitted Party will notify RCGM/GEAC immediately by telephone and positively within 24 hours in writing. RCGM/GEAC will provide the Permitted Party with the appropriate course of remedial action.

#### **D.7 Record Keeping**

D.7.1 The Record of Harvest/Termination must be completed by the Trial In-Charge immediately after harvest or termination of the contained trials at a trial site. This record must be verified and signed by a member of the Monitoring Committee or any nominee of RCGM/GEAC authorized by RCGM/GEAC to conduct a trial site inspection during harvest.

D.7.2 A copy of the Record of Harvest/Termination must be submitted to RCGM/GEAC within 15 days of harvest/termination of contained trials at the trial site. One copy is to be retained by the Trial In-Charge and one copy will be submitted to, and retained by, the Permitted Party

D.7.3 All records associated with the harvest or termination of contained trials must be available for inspection by RCGM/GEAC, State Government Officials, or their nominee upon request.

D.7.4 At the end of the post-trial activities when all requirements for management of the contained trial site have been completed, the original copies of all reports related to conduct of the trial will be forwarded to the Permitted Party.

D.7.5 The following records of all permitted contained trials for a minimum of five (5) years, whether or not the regulated GE Silkworm hybrid is authorized for commercial release, have to be made available (archive copies) by the permitted party:

- i. Record of Harvest/Termination
- ii. Record of Corrective Action (if applicable)

**5. CORRECTIVE ACTION IN THE EVENT OF AN ACCIDENTAL RELEASE**

5.1 In the event of a confirmed accidental release of regulated, GE silkworm eggs or live silkworm material during the trial, all attempts shall be made to recover as much of the regulated material as possible. Recovered material must be rendered non-viable and disposed of by heating, crushing, or burning.

5.2. The location of an accidental release must be marked and monitored to ensure that any progeny arising from the regulated GE silkworm eggs are rendered non-viable and disposed of by heating, crushing, or burning. The period of monitoring will be determined in consultation with RCGM/GEAC.

5.3 The incident will be immediately documented in a Record of Corrective Action. The original Record of Corrective Action is to be retained by the Recipient (Trial In-Charge) and copies will be submitted in writing preferably by fax/ email to the supplier (Transport In-Charge), Permitted Party and RCGM/GEAC.

5.4 Any other corrective actions will be determined in consultation with RCGM/GEAC.

**6 REVIEW OF SOPs**

These SOPs will be reviewed by RCGM/ GEAC at least annually.

**7 DECLARATION TO BE GIVEN BY THE TRIAL IN-CHARGE**

<b>DECLARATION</b>	
I hereby declare that the transgenic silkworm material issued to this centre will be used only for the purpose of evaluating their performance in the designated area under the supervision of the undersigned. These transgenic material –	
A. Will not be used in mating with any other transgenic / non-transgenic material	
B. Will not be distributed to non-designated areas for rearing	
C. Will not be given to any unauthorized persons	
D. Will not be put to any use other than those specified	
E. Will not be used for commercial cultivation	
Date:	Signature of the Officer-in-charge of transgenic trials



### Brief note on the development of BmNPV resistance transgenic silkmoths

Silkworms are affected by a number of diseases due to infection by pathogens and pests like virus, bacteria, microsporidia, insect etc. *Bombyx mori* Nucleo Polyhedrosis Virus (BmNPV) causes around 40% of the total cocoon crop loss. Although, the advances in many conventional practices have brought in significant impact on qualitative and quantitative improvements in silk production, incomplete understanding of molecular events in baculoviral life cycle *in vivo* and inheritance pattern of viral resistance of host strains makes it difficult to breed silkworm strains that are resistant to baculoviral infection.

In view of the limitations in conventional breeding, use of alternate strategies for introducing anti-viral property to the silkworm is required. Scientists at CDFD made use of biotechnology tools to successfully develop silkworm strains that are resistant to baculoviral infection. A brief description of the technology is described below:

#### Methodology

Transgenic silkworm lines expressing dsRNA for multiple essential viral genes were generated by *piggyBac* mediated germline transgenesis. *piggyBac* vector expresses dsRNA of four multiple essential baculoviral genes in an inverted-repeat arrangement under cytoplasmic actin (*BmActin*) promoter was used for transformation of Nistari strain. A DsRed gene under 3XP3 promoter enabled identification of transgenic lines by their red eye fluorescence. To assess the efficacy of these lines, transgenic silkworms were then subjected to *BmNPV* infection at a rate of 40,000 occlusion bodies (OBs) per larvae. The survival rate as well as cocoon quality traits were evaluated. Copy number, site of insertion of transgenes and their chromosome locations in different transgenic lines were identified and shown. Quantitative PCR analysis using viral genes (*lef3*) as a target gene showed reduced viral load in the transgenic lines.

The antiviral property of the Nistari transgenics was transferred to a baculovirus susceptible, bivoltine, commercial silkworm CSR2 strain through transgene selection coupled with microsatellite marker-assisted screening and repeated backcrossing. The introgressed transgenic silkworm lines showed ~80% resistance as compared to <15% resistance in control lines against *BmNPV*. The transgenic Nistari and CSR2 lines were crossed with commercial breeds of India to generate transgenic hybrids. Transgenic lines have commercial silk and cocoon traits (~1.8 gms cocoon weight; >1000 m filament length), and also resistance property of the parental line. The performance of various transgenic hybrids has been extensively studied at APSSRDI.