Suggestions for SOPs Submitted for APHIS BRS Permits

1.0 General Suggestions

Biotechnology Regulatory Services (BRS), a program within the Animal and Plant Health Inspection Service (APHIS) of the United States Department of Agriculture (USDA), offers these suggestions for the preparation and submission of a standard operating procedure (SOP) for applications to import, move interstate, and/or release into the environment organisms developed using genetic engineering (modified organisms) that are subject to the regulations under 7 CFR part 340.

To obtain an APHIS BRS permit, the responsible person creates a permit application and an associated SOP which are submitted and certified in the APHIS eFile electronic permitting system. The APHIS eFile system will not accept an APHIS BRS permit application unless at least one document with the attachment type of "SOP" is uploaded to the SOP/Attachments section of APHIS eFile.

The purpose of the SOP is to provide a description of how the modified organism will be

- **contained** during movement and at the points of origin and destination, including intermediate destinations, and
- **confined** during release into the environment "in a manner so as to prevent its unauthorized release, spread, dispersal and/or persistence in the environment" (7 CFR § 340.5(i)(1)).

Most permit applications that APHIS BRS receives are for modified plants, so consequently many of the examples provided throughout this document pertain to containment and confinement of plants. Those of you submitting applications for modified non-plant organisms should apply the general concepts of containment and confinement to the specific biology of your non-plant organism. The procedures proposed in the SOP should be sufficient for APHIS BRS to determine that containment

and/or confinement will be adequate according to the biology of the modified organism, its proposed proximity to sexually compatible species, the genotype and phenotype resulting from the modification, and the type of movement (importation, interstate movement, and/or environmental release). Important definitions follow:

- A secure shipment is a shipment in a container or a means of conveyance of sufficient strength and integrity to withstand leakage of contents, shocks, pressure changes, and other conditions incident to ordinary handling in transportation.
- A contained facility is a structure for the storage and/or propagation of living organisms designed with physical barriers capable of preventing the escape of the organisms. Examples include but are not limited to laboratories, growth chambers, fermenters, and containment greenhouses.
- Confinement is a collection of activities and site attributes meant to prevent the unauthorized release, spread, dispersal, and/or persistence in the environment of plant pests or organisms developed using genetic engineering that pose a plant pest risk.

Information provided in an SOP may be used by APHIS BRS to establish supplemental permit conditions to ensure adequate containment and confinement (7 CFR § 340.5(i) *Permit conditions*). **The content of the SOP should be limited only to the information needed to describe containment and confinement and must be specific for the organism and the locations of importation, interstate movement, and environmental release.** For example, if your modified organism is a plant and no plantings are planned outside the continental United States, do not add information for plantings in Hawaii or Puerto Rico. Furthermore, if your application proposes only one or two of the three movement types and you submit a comprehensive SOP that includes sections for all three movement types, APHIS BRS may reject your SOP or review only the section(s) that apply to the movement type(s) applicable to your permit application. An exception is if the comprehensive SOP has been pre-reviewed by APHIS BRS.

1.1 Format for the Standard Operating Procedure

The information in the SOP may be in a format that the responsible person chooses; APHIS BRS offers some suggestions for naming and formatting below. The Design Protocols for APHIS BRS permits submitted prior to April 5, 2021, will not be accepted in eFile starting April 5, 2021. However, Design Protocols can be revised as SOPs that meet the requirements of the modern biotechnology regulations in 7 CFR § 340.5.

Use a unique and descriptive filename for your SOP and other attachments for ease of identification in the eFile folders and to help avoid confusion. APHIS BRS recommends that your SOP filename use the following naming convention:

<Organization Name>_<Organism Common Name SOP>_<Movement Type>_<Date>.<docx or pdf>. The organization name can be abbreviated, and the date given in the ISO international date format yyyy-mm-dd. For example: ABC Univ_Pepper SOP_Release_2021-04-05.pdf.

A descriptive document heading and/or introductory paragraph containing the elements below (broken out in bullets for example purposes) can facilitate review and compliance activities:

- Organization name
- Standard Operating Procedure for the type of permit, whether it is an importation, interstate movement, and/or environmental release, with common name, version number, and date (e.g., Standard Operating Procedure for Environmental Release of Pepper, V1, April 5, 2021)
- Name of the Responsible Person or their Agent who maintains control of the modified organism and ensures compliance with the permit conditions
- Plant scientific name (genus and species) and name of subspecies or variety or cultivar when applicable (e.g., *Capsicum annuum* var *annuum*)
- Plant common name and ecotype (e.g., cayenne pepper, spring or winter wheat, upland or lowland switchgrass, etc.)
- Country and locality where the organism was collected, developed, manufactured, reared, cultivated, and cultured (as applicable)(e.g., ABC University Capsicum Germplasm Collection, Las Cruces, NM).

If the SOP has confidential business information (CBI), you must submit an accompanying CBI-deleted version. Further information can be found in the Guidance for Claiming Confidential Business Information (CBI) in Submissions to USDA APHIS Biotechnology Regulatory Services at https://www.aphis.usda.gov/brs/pdf/CBI_Submission_Guidance.pdf.

The SOP may be submitted to APHIS BRS for a pre-review in advance of your permit submission. To exercise this pre-review option, email your SOP to BRS.Permits.SOP@usda.gov ***at least 60 days*** before you intend to submit your permit authorization in eFile. You should plan to submit your permit authorization ***at least 45 days*** before you intend to begin your importation or interstate movement, or ***at least 120 days*** before you intend to begin your environmental release, in accordance with APHIS BRS administrative actions for approval or denial of a permit (7 CFR § 340.5(h)(5)).

The SOP submission can be encrypted with a password if it contains CBI. Once an SOP has been pre-reviewed and acknowledged by APHIS BRS, the acknowledged version can be uploaded as a PDF to all the applications for that species as long as the procedures in the SOP are applicable to the movement type and phenotype/genotype described in the permit. If you make revisions to an acknowledged SOP, you must enter a new version number and date in the revised SOP Word document, and add a brief comment in the Additional Information section of the application explaining that the SOP has been revised since it was last acknowledged by APHIS BRS.

1.2 Uploading SOPs to an Application in eFile

To upload an SOP and other attachments to applications in eFile:

a. Select the “SOP & Attachments” stage at the top of the Standard Operating Procedure & Attachments section of your application to see instructions on how to prepare and add an SOP to the application.

b. To upload the SOP and other attachments to the line item, scroll past the imbedded instructions and click on the green button "Add SOP or Attachment"; this will open a new tab where details about the type of document uploaded to the application can be added.
c. There is a choice to drag and drop the files onto the page or use the "Select a File" button to choose a file from the document library on your computer.
d. There are two required "picklists" or drop-down menus. The "Attachment Type" picklist allows for selection of the type of attachment to be uploaded. A "CBI Version" picklist allows for the selection of the CBI status of the document with three choices: CBI Included, CBI Deleted, and No CBI. If an SOP containing CBI is uploaded, a CBI-deleted version must also be uploaded to the application.
e. Once the documents have been uploaded, press save, and the window will close automatically after a successful upload.
f. To view the uploaded document, click on the folder icon in the top left corner of the screen/page to refresh the page.

2.0 Importation or Interstate Movement

The information in the SOP for an importation or interstate movement permit should describe how the responsible person will comply with the regulations (including 7 CFR § 340.5(b)(2), § 340.5(i), and § 340.5(m)) to ensure that their modified organism is securely labelled, packaged, shipped, stored, segregated, and disposed of in a manner that will prevent the organism's unauthorized release, spread, dispersal, and/or persistence in the environment.

2.1 Packaging and Shipping

Consider how organisms will be contained so that dissemination of viable material during movement is unlikely, taking into account propagule size, container volume capacity, and shipping vehicle security. Inclusion of information such as described below is helpful to show that the modified organisms and accompanying biological material will be contained:

- Describe any other material such as biological material used to support the modified organism during importation or interstate movement, e.g., potting mix, soil, or culture medium.
- Describe how the modified organism or parts thereof are enclosed in at least two independent layers of packing materials so that if one layer fails, there still will be an independent layer of containment.
- Describe the packing materials and, if applicable, how they are resistant to and protected from deterioration due to exposure to the external environment, how they are sift-proof (impermeable to dry contents, including fine solid material produced during transportation), and how they are water-resistant so that the regulated material will be contained during shipment and not escape into the environment.
- Describe that the container or package used in transportation will be accompanied by a document that includes the names and contact details of the sender and recipient.
- Describe how packaging material, shipping containers, and any material accompanying the organism will be cleaned and disposed.

Examples of acceptable devitalization methods for packaging materials are autoclaving, incineration, or treatment with bleach. All modified organisms or parts thereof, such as seeds, that result from cleaning the packaging material or shipping containers must be collected and devitalized. Collection of the modified organisms remaining in packaging material can be done by sweeping, vacuuming, or washing with water hose/jets, for example.

2.2 Storage, Identification, Segregation, and Labeling of Modified Plants

For compliance with 7 CFR § 340.5(i)(1)-(6), the modified organism under permit must be:

- Maintained and disposed of in a manner to prevent its unauthorized release, spread, dispersal and/or persistence in the environment
- Kept separate from other organisms, except as specifically allowed in the permit
- Maintained only in areas and premises specified in the permit
- Maintained so that they are always identifiable and verifiable
- Authorized activities may be engaged only while the permit is valid
- Maintained with records of sufficient accuracy, quality, and completeness to demonstrate compliance with the SOP and the regulations.

Consider describing in the SOP procedures for:

- Identification of the modified organism under permit at the origin and destination(s), so that the identity of the modified organism is always clear and verifiable.
- Maintenance of organism under permit so that it is kept separate from other organisms, except as specifically allowed in the permit.
- Maintenance of regulated material, such that the material in a contained facility or in a storage container or cabinet in a contained facility, is secured by a lock or other method with access only to authorized personnel.

Containers may be of different sizes and materials, including bags made of paper and other materials; modified plants or viable plant products can be kept in a locked drawer, cabinet, cold rooms, or other storage facilities. Larger quantities may be stored in secure field containers or silos.

Example: A plastic container closed with a secure lid and labeled with waterproof tags, kept in a locked cabinet, that is not used for non-modified plants or viable plant plants.

3.0 Environmental Release

For compliance with 7 CFR § 340.5(i)(1)-(6), the modified organism under permit must be:

- Maintained and disposed of in a manner to prevent its unauthorized release, spread, dispersal, and/or persistence in the environment
- Kept separate from other organisms, except as specifically allowed in the permit
- Maintained only in areas and premises specified in the permit
- Maintained so that its identity is always verifiable
- Authorized activities may be engaged in only while the permit is valid

- Maintained with records of sufficient accuracy, quality, and completeness to demonstrate compliance with the regulations and other requirements.

The SOP should describe the proposed activities and procedures that will be undertaken to prevent unauthorized release, spread, dispersal and/or persistence in the environment of the modified plant and/or plant parts under permit. The SOP should describe proposed procedures for containment during transportation to and from the field trial location; confinement during the environmental release from planting to harvest; termination of the field trial, and completion of volunteer monitoring.

3.1 Identification of the Release Site and of the Modified Plants

All plants within the field trial under permit are to be treated as regulated. The SOP will describe the methods used for identification of the modified plants and of any other plants that are part of the field trial. GPS coordinates must be provided in the permit and the SOP will describe how the trial will be marked on the field. Also describe how access by unauthorized persons and animal incursions will be prevented.

The release site and plants can be identified with stakes, markers, GPS coordinates and associated maps, physical barriers, and distinct border crops. GPS coordinates must be provided in the permit, the SOP must describe how the trial will be marked on the field.

3.2 Confinement to Prevent Unauthorized Release, Spread, Dispersal, and/or Persistence

Procedures for confinement of the modified plant within a field trial take into account the biology of the plant, including how the plant reproduces; pollination method (e.g., wind, insect, self-pollinating); the distance that viable pollen can travel; propagule type and size (e.g., seed, tubers, cuttings, other); how propagules are dispersed (e.g., wind, shattering, animals); dormancy of seeds and other reproductive structures; and environmental persistence. Canopy size may need to be considered if the modified plant is a tree. The presence and proximity of sexually compatible relatives within the reproductive isolation distance should also be considered, including whether they are from another crop or from wild and/or weedy relatives.

Things to consider in the SOP for **containment/confinement during field operations:**

- Containment during transportation of propagules from storage to field location for planting, and from field to storage location after harvest.
- Use of a **perimeter zone** (or separation distance) that is at least 10 ft wide for confinement of modified plants within the field trial site, to avoid mixing with plants in adjacent fields that are not part of the field trial, during field operations.
- The perimeter zone can be fallow or planted with a low growing ground cover of a non-sexually compatible, morphologically distinct species that allows for the detection of regulated material.

Reproductive Isolation suggestions to avoid gene flow from the modified plant to sexually compatible species not part of the environmental release:

- **Reproductive isolation distance** in feet between the fertile modified plant in the field and any sexually compatible species. Consult the Separation Distance Table on the BRS website.
- **Border rows** of the same species as the modified plant, to create a continuous perimeter of synchronously flowering plants intended to attract pollinators, when outcrossing occurs via pollinators.
- **Physical isolation** of flowers during the entire flowering period of the modified plant, such as bagging, caging, use of tunnels made of materials that do not allow movement of insects and pollen in or out or the tunnel.
- **Removal of flowers before anthesis**, including detasseling, emasculation, and mowing the plants to prevent flowering.
- **Male sterility** when plants have been verified to be incapable of producing male flowers before the release. This is not acceptable as the only method of reproductive isolation for some species, and an additional method should be described to ensure confinement.
- **Temporal isolation** when the timing of planting of the modified plant is such that flowering is asynchronous with flowering of any sexually compatible species within the pollination distance. This is typically based on planting dates or growing degree days or heat units and needs to be documented. Monitoring is also needed to ensure that there is no outcrossing from the modified plant to sexually compatible relatives.

3.3 Termination of the Field Trial and Devitalization

The SOP should include a description of procedures proposed to terminate the field trial and devitalize the modified plants so that no viable modified plants will remain in the field to grow, persist, or reproduce in subsequent growing seasons. In addition, if modified plants are harvested, you should describe how the materials will be secured in a contained facility and devitalized when no longer in use. Some examples of devitalization methods are lethal desiccation, autoclaving, incineration locally or at another facility, and deep burial to at least 0.6 m (2 ft). You should describe at least one alternative method in case the preferred method cannot be carried out.

3.4 Monitoring to Prevent Unauthorized Release, Spread, Dispersal and/or Persistence

In-Season Monitoring must be appropriate for the reproductive isolation method in use for the field trial, and the responsible person or their agents must maintain control of the modified plants for the duration of the permitted activities. In-season monitoring examples are given below:

- **Reproductive isolation distance:** monitor within the reproductive isolation distance for the presence of sexually compatible relatives that are not part of the release.
- If **border rows** of pollen trap plants are used, monitor throughout the flowering period of the modified plant to ensure synchronous flowering and that there are no significant gaps in the border row.
- **Physical isolation:** monitor to ensure continuity of isolation every one to three days for the duration of flowering.
- **Flower removal:** monitor prior to flower emergence until flowering ends and frequently enough to remove flowers from side shoots or suckers that may emerge later.

- **Temporal isolation:** frequency and duration of monitoring for flowering in the modified plant and in sexually compatible relatives within the isolation distance must be described and then documented as it occurs.
- A **contingency plan** should be described in the event that the reproductive method fails or is likely to fail, such as if cages or bags are lost or breached or temporal isolation fails.

Volunteer Monitoring (VM) After Termination of the Field Trial must be described and be appropriate for the reproductive biology of the plant and dormancy period of the reproductive structures. The monitoring area will include the planted area and the perimeter zone. The monitoring area may be extended if there is a breach in reproductive isolation. You should consider the following when describing in the SOP the procedures used for volunteer monitoring:

- Describe the frequency and duration of monitoring, considering the growing season for the modified plant in the area where the environmental release will occur.
- State the time frame when volunteers will be devitalized; typically, volunteers are devitalized upon discovery or sometime before reproductive maturity.
- Describe the method used for devitalization of volunteer plants.

3.5 Replanting after Field Trial Termination and During Volunteer Monitoring

If the field will be replanted after field trial termination, the SOP should address the detection, identification, and removal of volunteers of the modified organism and progeny prior to anthesis and prior to any grazing or the harvest of any crop for food, feed, or seed.

- Replanting to the same or sexually compatible unregulated crop is generally not allowed until sufficient time has passed to control all volunteers effectively.
- Acceptable management or replanting options during volunteer monitoring typically include:
    - Leaving the site fallow
    - Planting to another **morphologically distinct, non-sexually compatible crop which allows for the detection of volunteers**
    - Planting to another **morphologically distinct, non-sexually compatible cover crop** for erosion control that is plowed under
    - Replanting the **same regulated crop in a regulated trial** when the volunteers are subject to the confinement methods employed for the regulated crop and the latter trial is monitored for volunteers.