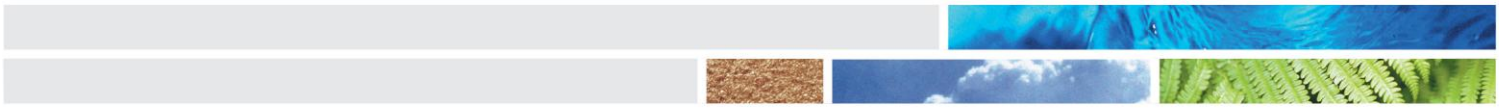
Environmental Protection Authority
Te Mana Rauhī Taiao

Appendices to the EPA staff advice for applications ERMA200706 and ERMA200792 – the development and import of *Arabidopsis thaliana* in containment

JUNE 2012



APPENDICES

New Zealand Government

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Appendix 1: *Arabidopsis thaliana* containment guidance document ver. 2.

1.1. The applicants' containment protocols and guidance document address pathways of escape for *Arabidopsis thaliana* plants and seed (Appendix 4). These include policies and procedures that deal with design, construction, and maintenance of the containment facility; waste water treatment; training of facility users; record keeping; regular reviews of containment protocols; contingency plans in case of an emergency, or in case of a containment breach; and transport and destruction of approved organisms.

1.2. The guidance document will be used by each applicant institution to develop a facility-specific containment manual. The containment manual will detail such matters as the physical and procedural measures put in place to maintain compliance, requirements for staff training, and the appointment of staff members to oversee compliance with the controls. The manual will be required for Ministry for Primary Industries (MPI) inspectors to approve the containment facility under section 39 of the Biosecurity Act 1993. The manual will be regularly reviewed when monitoring the facility to ensure all protocols are being followed and containment of *A. thaliana* is maintained.

1.3. After the external review of the guidance document by the expert panel from New Zealand and an international expert we incorporated a few modifications to the document to reflect their feedback.

Arabidopsis thaliana Guidance Document ver. 2:

This document is intended to help establish best practice policies when containing the HSNO approved organism Arabidopsis thaliana – to be developed or imported. This is guidance to meet the controls imposed by the approvals ERMA200706 and ERMA200792.

Controls

Each of the applicant organisations granted approval under this decision (each referred to as the approval holder) must ensure compliance with the controls set out in respect of any work they carry out under this approval in a facility under their control

Requirements for containment

1. The approved organism (Table 1) must not escape containment.
2. This approval is limited to the development in containment of the approved organism.

[Alternative 2. This approval is limited to the importation of the approved organism into containment.]

3. A management plan must be documented specifying procedures for implementing the controls imposed under this approval.

Guidance note: The applications include whole plants that may form reproductive structures. Seeds are heritable material. Because *A. thaliana* is self-pollinating, extra consideration for containment of pollen is not warranted. Imports may include *A. thaliana* cell or tissue cultures into containment but we would like to note cell/tissue cultures cannot survive without human intervention, and can not establish in the environment.

It is recommended that the current approval users' containment facility manual be updated to record how each control will be met. This guidance document and other guidance material (such as The Australia/New Zealand Standard: Safety in Laboratories 2243.3) should be used for reference.

Requirements for containment facility

4. The containment facility must be designed and constructed to contain the approved organism held within it.

Guidance note: The Australia/New Zealand Standard: Safety in Laboratories 2243.3 and AS/NZS 2982 Laboratory Design and Construction are both invaluable resources for design assistance. It is strongly recommended that advice should be sought from MPI at the time of construction. MPI should be informed at all stages of design and construction and at the time of any alterations to the facility.

Facilities will continue to be approved under the Biosecurity Act 1993.

In light of the organism to be contained, it is the seed that has the highest potential risk of escape, therefore we have highlighted structural elements designed to reduce the potential of seed to escape the facility:

- **The junction between floor and wall in containment glass houses/facilities should be sealed.** This might be achieved a number of ways, including by a sealed concrete nib wall which is mortared to the floor pad, or in the case of internal plant facilities, by covered flooring.
- **There should not be any gaps under doors.** This could be achieved through bunding, or a raised door entrance to prevent seed being blown under the door. Alternatively, the door could be fitted with brushes, but attention should be given to the capture of seed in the unlikely event that seed makes its way through the brush barrier.
- **Where drains are present care is needed to prevent seed being washed down the drain. It is also important that drains do not provide a point of entry for rodents and insects**.

It is suggested that drains are screened with fine mesh (the size of which should reflect the size of *A. thaliana* seed), or a wad of filtration material (such as F6 air filtration matrix) that can be removed when clogged. This filtration material can be autoclaved, disposed of, and easily replaced. Regular checks of the mesh and/or filtration material should be carried out as part of routine maintenance.

- **All waste water should be managed to ensure it contains no live approved organisms or heritable material.** Water management practices may include the use of disinfectants for small volumes of water (up to 50 litres) to render any seeds non-viable (note: disinfectants will need to be very aggressive to render the seed non-viable), or filtering to prevent the escape of any live plant material, taking into consideration the size of seeds. The water can then be discharged into the municipal sewer systems. Filters should routinely be changed and waste material disposed of as per approved waste disposal procedures. These methods are only indicative, for example the use of water traps and UV light to render any seeds non-viable can be used.
- **A freestanding containment facility should have an anteroom for entry and exit. An anteroom is not necessary if the facility connects directly to a certified containment facility.** Facilities may consider an anteroom that has air pressure different to the containment facility, to ensure any seed will be blown back into the containment facility. This will also prevent easy entry of unwanted contaminants and insects.
- **Flooring material should be smooth, seamless, and impervious to allow easy capture of seed.** It is suggested that the use of a vacuum cleaner (fitted with a HEPA filter) can reduce or remove the need to use water on floors.

Note: Notwithstanding the need for proper containment facilities, the primary emphasis on containment of *A. thaliana* seed has to be on seed capture by primary and secondary containers (such as bagging of seed heads and large trays).

5.	**The containment facility must be maintained in order to contain the approved organism held within it (i.e., preventing escape).**

Guidance Note: This control refers to the physical maintenance of the containment facility. All regular users of the facility should be instructed to recognise and report problems.

- **It is highly recommended that an experienced staff member be appointed to coordinate maintenance, and a regular schedule should be in place to monitor the facility, and to check/clean filters, and perform other necessary maintenance.**
- **All users should be trained to recognise and immediately report problems or potential problems that may result in a breach of containment.**

6.	**All measures must be taken to prevent the accidental or deliberate release of an approved organism from the containment facility.**

Guidance Note: This control reflects the procedural policies that needs to be in place to contain *Arabidopsis* in the facility.

- **It is essential that all plants housed in the plant house should be considered and treated as new organisms. Therefore, containment policies should be implemented for all plants in the facility.**
- **A central register is highly recommended. This should demonstrate that an organism is part of the HSNO approval, and will allow tracking of organism (plant/seed) provenance.**
- **The use of easily cleaned large trays under plants (including at sowing stage) is crucial. The trays should be large enough to extend well beyond the spread of the plant to ensure that any seed falling from bent stems will be captured.**
- **Bagging (or a similar measure) of seed heads is an a important primary containment measure, but plants should also be on larger trays which act as secondary containment and prevent seed falling to the floor where is it less likely to be contained.** Note*:* Containment of seed is the most important primary containment measure. However, if bagging of seed heads is premature, there is a risk of fungal infection resulting in loss of important research material and promoting over-planting. It is recommended that bagging (or a similar measure such as growing in high sided containers or aracons) is undertaken when the siliques turn yellow. Plant house staff should be instructed to bag plants where necessary to ensure the seed head will always be bagged. Alternatively, the plants can be grown in high sided containers to prevent seed falling onto the floor from seed heads. It is also best practice to position plants away from any strong airflow from air-conditioning units.
- **Dedicated laboratory coats for use only in the plant house are required.** It is preferable that these lab coats should be pocketless, which prevents seed inadvertently escaping via the pockets. It is also recommended that disposable shoe covers or dedicated shoes should be worn in the plant house (option) to prevent the escape of seed.
- **Sticky floor mats supplied for clean rooms are helpful, especially for indoor facilities.**
- **Dedicated wet and dry vacuum cleaners are also helpful. These should be fitted with a HEPA filter, and the waste contents from the vacuum cleaner should be autoclaved before disposal.**
- **It is suggested that containment protocols are reviewed annually, and that all technical and research staff are involved in the review.** This ensures all containment protocols are current, workable and take into account any incidents that indicate potential failure of containment measures.

- **Users may consider the use of artificial growth media.** Artificial media is lighter, does not harbour mites, is more easily transported, and more easily autoclaved and disposed of than soil.
- **Plant house procedures may include a regular herbicide spraying regimen around exterior plant houses, to maintain a clear area around the planthouse.**
- **Plant house procedures may include a regular insecticide spraying regimen to kill arthropods, especially mites.**

Requirement for entering/exiting containment facility

7. All measures must be taken to ensure that persons entering and exiting the containment facility do so in a way that does not compromise the containment of the approved organism.

Guidance note:

Procedures should be in place to ensure all users of the facility (including any new or short-term users) are aware of the controls required by this approval.

- **All users need to be trained in the procedures required by this approval.** It is recommended that the facility manager should ensure that only those persons who have *bona fide* scientific research requirements and who have been properly instructed are permitted access to the facility. Management staff should not be given default access unless they are properly instructed and are aware of containment measures.
- **Contractors, trades people, and visitors may only be given access to the facility after adequate instruction.** It is suggested that visiting trades people should be required to sign a simple sheet indicating they are aware that the plant facility is for the containment of plants and seeds, and they will observe all entry/exit procedures, will not come in contact with plants, and will ask trained personnel to move plants if required. Trades people should also be instructed to inform the plant house technician when work has been completed and their exit should be documented.
- **Maintenance staff should be instructed in case of emergency.** The tradesperson induction should include a requirement to contact the plant house technician in the event of an emergency.

Requirements for moving new organisms

8. All measures must be taken to prevent the escape of an approved organism during any movement within the containment facility or outside the containment facility.

Guidance note:

- **Double containment is required for transport between the plant facility and laboratories**. Any approved organism should be contained in at least two closed containers

for transportation. It is suggested that these could include plastic microcentrifuge tubes, plastic screw-top centrifuge tubes, or air tight plastic boxes.

- **Three layers of containment are recommended for transport outside of the containment facility.** The third layer includes the packaging material. For further guidance for packaging look to packing instructions from IATA.

9. Provided Control 8 is complied, with an approved organism may be moved from a containment facility to another containment facility for the purposes of laboratory based research, teaching, disposal, in case of an emergency, or any other necessary purpose.

Guidance note:

- **In the specific case of moving saved seeds to another containment facility for storage purposes and transported inside double closed containers** (see above). When stored, seeds should be maintained in a spill-proof, labeled container. Seed containers should clearly identify the provenance of the seed and its genetic background.

10. Containment measures for an approved organism being transferred must require the container holding the approved organism to clearly identify the contents, containment requirements, and the details of the sender and receiver.

Guidance Note: Stored seed containers should be clearly identify the provenance of the seed and its genetic background.

- **A log of all new organisms imported into, transferred out of, or produced within the facility should be kept**. This log should describe the new organism, and provide the HSNO Act import or development approval number, its location within the facility, or if it has been disposed of or transferred to another containment facility. It is sufficient to describe the organism at the experiment level.

Requirements to limit access to the containment facility

11. All entrances must clearly identify the facility as being a containment facility.

Guidance Note: Signs should be put up at all laboratory entrances indicating the area is a containment facility and access is limited to authorised personnel.

- **It is suggested that in addition to standard containment facility signage the additional requirements of a restricted plant house should be displayed.** These should include contact mobile telephone numbers for responsible persons, and requirements for trades people and facilities management staff to contact the plant house technician (see Guidance note for control 8).

12. All personnel entrances and other means of access into the containment facility must be specified in the management plan.

Guidance Note:

- **Up to date floor plans are the clearest method to indicate where all the entrances to the containment facility are located. Plans should be kept with Containment Facility manual.**

13. Unauthorised persons must be excluded from the containment facility.

Guidance Note:

- **All doors should be lockable and locked when not in active use.**
- **Contractors and trades people should only be given access after receiving adequate instruction.** See guidance note for control 7.
- **Management staff should not be given default access to the facility unless they are properly instructed and aware of containment measures.**

Requirements for removing equipment and waste from the containment facility

14. 14. Any waste (including biological material) that may harbour the approved organism, or heritable material from the approved organism, must be treated to ensure that the approved organism and any heritable material is killed prior to its removal from containment.

Guidance note:

- **Autoclaving is the preferred method of treatment to kill or neutralise waste or heritable material from an approved organism.** Other treatment methods may include the use of chemicals or incineration. Researchers should consider using artificial growth medium instead of soil when growing *A. thaliana* plants. Medium is much lighter and more easily contained than soil, making transport to and from the autoclave less problematic.

15. Any equipment that may harbour the approved organism, or heritable material from the approved organism must be treated to ensure that the approved organism and any heritable material is killed prior to being used for another purpose or removed from the containment facility.

Guidance Note: Treatment of equipment to remove any approved organisms or heritable material can include treatment of any parts of the equipment that come in contact with the approved organism or heritable material, and that can effectively be treated.

Requirement for training

16. All persons entering the containment facility (including contractors, staff, students, visitors and volunteers) must have received instruction on the containment practices of the containment facility relevant to the responsibility of the individual.

Guidance note:

- **All training should be documented.** All staff, students, and researchers working within the facility should be instructed on the operational procedures for containment. All tradesmen, contractors, visitors and volunteers entering the facility should be accompanied by staff or instructed on containment procedures, relative to their responsibility. See guidance note on contractors above (control 8).

- **Ongoing refresher training is critical**. Regular users of the facility should be involved in regular periodic reviews of containment procedures and required to report any potential breach of containment.
- **Training should include (but is not limited to):**
  - HSNO controls on the approved organism
  - Possible pathways of escape of the approved organism, and the procedures in place to maintain containment.
  - Organisational risk management associated with maintaining containment
  - Contingency plans.

Requirements for contingency plans

17. The management plan must contain contingency plans for the accidental release or escape of an approved organism. The plan must:

- **describe the activities that will be implemented in the event that containment of an approved organisms is compromised or potentially compromised;**
- **include a description of recapture and eradication protocols;**
- **be able to be implemented for each approved organism; and**
- **be implemented if there is reason to believe that an approved organsm has escaped or been released from the containment facility.**

Guidance note:

- **Contingency plans should be a part of any training that occurs to educate staff on policies**.
- **Discussion with your MPI inspector on follow up procedures is important.** Ensure that within 24 hours of the discovery of any breach of containment[1], the MPI Inspector responsible for supervision of the facility, has received notification of the breach, and the details of any action taken by the facility since the breach occurred.
- **Plans should also reflect when a decision should be made to destroy the plants to guarantee they will not escape.**
- **It is recommended that contingency plans take into consideration that many emergencies will affect communications and electricity supply.** This may not happen immediately, but some time after the emergency (eg. cell phone towers have a short battery life). Provided the structural integrity of the facility is intact, a contingency plan may include closure of the plant house to prevent entry until essential services are returned to normal.

[1] Breach of containment includes: the escape of an organism(s), unauthorised entry and/or structural integrity of facility compromised.

- **The facility should have documented contingency plans that are to be implemented should there be a suspected release or escape from containment.** The facility needs to include means to implement the contingency plan, including but not limited to an effective herbicide.

Requirements for inspections and monitoring

18. To ensure containment is being achieved and to identify any remedial maintenance requirements each containment facility must be inspected by the approval holder at reasonable intervals given the nature of the approved organism being contained.

Guidance Note:

- **Regular maintenance programs should be developed, including internal audits, and routine maintenance checks, etc.** The responsibility is on the organisation to ensure audits (in addition to monitoring by MPI) are conducted regularly so that any faults in containment are remedied as soon as possible. All users of the plant house should be involved in surveillance through the reporting of faults or potential problems. Records should be kept of inspections and cleaning etc, to allow tracking of the inspection and encourage regularity in this process.

19. The approval holder must grant MPI[2] access to the containment facility and relevant documentation for the purpose of auditing and inspecting.

20. Each containment facility must be inspected by the approval holder as soon as possible after any event that could compromise the containment regime, such as an Act of God (eg. flood, earthquake), or any unauthorised attempt to enter the containment facility.

Guidance note:

- **Keep in regular contact with your MPI inspector as soon as possible to update them on conditions and status.**

21. Remedial containment requirements identified by the approval holder or MPI (as applicable) must be completed as soon as possible, including such interim measures as are necessary to mitigate the risk of breach of containment.

Guidance note: This might mean approved organisms may need to be moved to a separate area within the containment facility, or to another containment facility.

- **Saved seeds may be moved to another containment area on the same institutional site for storage.** Seeds should be maintained in a spill-proof, labeled container.

[2] Ministry of Agriculture and Forestry (MAF) has been renamed the Ministry for Primary Industries (MPI). They are still the compliance and enforcement officers for the EPA, as empowered by the HSNO Act.

- **This might mean increased inspection rates, or spraying of herbicide, as outlined in the contingency plan.**

22. Any structural modifications to a containment that may affect the integrity of containment must be approved by an MPI Inspector prior to being used to contain the approved organism.

With reference to Table 1: The approved organsim

Restrictions on Genetic Modification

The applications for *Arabidopsis thaliana* import and development state that modifications to *A. thaliana* will not involve:

- the production of infectious particles;
- genetic material derived from Māori;
- genetic material derived from native flora and fauna;
- genes encoding known or predicted vertebrate toxins;
- uncharacterized sequences from pathogenic microorganisms;
- genetic modifications that increase the pathogenicity, virulence, or infectivity of the host organism; or
- genetic modifications that result in the modified organism having a greater ability to escape from containment than the unmodified host.

Guidance Note: These restrictions are imposed to ensure the modifications to *A. thaliana* under these import and development approvals are consistent with the HSNO Low-Risk Genetic Modification Regulations 2003. If you plan to develop or import *A. thaliana* with modifications that fall outside these exclusions, or which are ambiguous, you may require a separate HSNO application. For further clarification you should consult with the EPA in the first instance (email:noinfo@epa.govt.nz).

- The term ‘unmodified host’ refers specifically to the original wild-type strain of *A. thaliana*. Therefore genetic modifications must not result in the modified organism having a great ability to escape containment than wild-type *A. thaliana*. For example, gene complementation experiments are permitted.
- Care should be taken when planning experiments to test altered growth, productivity, and reproduction of *A. thaliana*. When planning these types of experiments, due consideration should be given to the expected effects. For example, modifications that increase seed production are not likely to increase the risk of escape; however, modifications that reduce seed size are likely to give the organism a greater ability to escape, and specific protocols for

the containment of seed may need updating. For further clarification on whether a proposed modification complies with these approvals, please consult the EPA (see above).

Appendix 2: Schedule 3 Part 1: Matters to be addressed by containment controls for importing, developing or field testing of genetically modified organisms

1. To limit the likelihood of any accidental release of any organism or any viable genetic material, the controls imposed by an approval shall specify:

(a) Requirements for treatment and decontamination to prevent escape by way of expelled air, discharge of water or liquid waste, removal of solid waste or goods, or breaches in facility boundary
(b) Equipment and requirements for facility construction to enable the requirements for treatment and decontamination to be readily met
(c) Requirements to be complied with for the access of persons to the facility
(d) Procedures and requirements for transport, identification, and packaging for all biological material to and from the facility and within the facility
(e) Requirements for the disposal of any biological material
(f) Requirements for facility construction
(g) Requirements to secure the facility and openings, including securing against failure in the event of foreseeable hazards

2. To exclude unauthorised people from the facility, the controls imposed by an approval shall specify:

(a) Means of identification of all entrances to the facility
(b) The numbers of entrances and access to the facility
(c) Security requirements for the entrances and the facility

3. To exclude other organisms from the facility and to control undesirable and unwanted organisms within the facility, the controls shall specify

(a) Monitoring requirements to establish the presence of other organisms
(b) Phytosanitary requirements
(c) Requirements to secure the facility and openings against likely unwanted organisms

4. To prevent unintended release of the organism by experimenters working with the organism, the controls shall specify

(a) Requirements to prevent the contamination of work surfaces, equipment, clothing, and the facility generally
(b) Requirements for laboratory practice to control infection by ingestion or breaks in skin cover
(c) Means to control infection by inhalation

5. To control the effects of any accidental release or escape of an organism

(a) Controls imposed by an approval shall specify an eradication plan for escaped organisms
(b) Controls imposed by an approval may specify requirements to limit the likelihood of an escaped organism spreading, surviving and breeding, including, but not limited to,—
  (i) Exclusion zones (spatial or temporal)
  (ii) Location of the facility outside the usual habitat range of the organism

6. Controls imposed by an approval shall specify inspection and monitoring requirements for containment facilities, including any inspection required before commencement of the development

6A. Controls imposed on an approval to field test a genetically modified organism—

(a) must specify—
  (i) inspection and monitoring of containment facilities during the field test; and
  (ii) inspection and monitoring of the site, after the field test, to ensure that all heritable material is removed or destroyed; and
(b) may specify inspection of the site before field testing commences

6B. Clause 6A applies, with all necessary modifications, to controls imposed on an approval to develop a new organism that is a genetically modified organism, to the extent that the development does not take place in a containment structure.

7. Controls imposed by an approval may specify

(a) The qualifications required of the person responsible for implementing the controls imposed by an approval

(b) The provision of a management plan specifying procedures for implementing controls imposed by an approval

Appendix 3: Effects pathway assessment of the proposed import and development of *Arabidopsis thaliana.*

1.1. These applications to import (ERMA200792) and develop (ERMA200706) genetically modified (GM) *Arabidopsis thaliana* in containment are jointly submitted by the University of Auckland, Massey University, University of Otago, Lincoln University, Scion, AgResearch Limited, ViaLactia Biosciences New Zealand Limited, and Canterbury University. Together they represent every organisation that currently holds an active HSNO approval to import or develop *A. thaliana* in containment.

Effect Pathways

1.2. In the assessment of a potential risk, we use effect pathways to determine the probability that the event has a consequence. We assume anything can happen, but consider that just because an event happens, does not mean there will be a consequence.

Biological characteristics of *Arabidopsis thaliana*

1.3. Though native to Europe, Asia, and North West Africa, *A. thaliana* is also present growing wild in New Zealand's environment (Webb et al, 1988).

1.4. *Arabidopsis thaliana* has the following biological characteristics that are relevant to the containment of the organism:

Ability of *Arabidopsis thaliana* to regenerate from plant material

1.5. *Arabidopsis thaliana* is not known to be able to regenerate (ie, grow) from plant material such as stems, roots or leaves. Therefore, the escape of *A. thaliana* from a containment facility and establishment in the environment through plant material such as stems, roots and leaves is not considered a feasible pathway.

1.6. *Arabidopsis thaliana* is not known to be able to regenerate (ie, grow) from plant tissue culture or cell culture material. Therefore, the escape of *A. thaliana* from a containment facility and establishment in the environment through cell or tissue culture material is not considered a feasible pathway.

1.7. We consider that whole plants being unintentionally removed from the containment facility by personnel in shoe treads, clothing or on equipment; in waste or waste water; by animals; during

transit between containment facilities; or deliberate removal by unauthorised personnel are feasible pathways of escape.

Ability of seed to escape the containment facility and establish new populations

1.8. *Arabidopsis thaliana* plants can produce up to 10,000 seeds per plant (Kimball, 2004). The seeds are released (ie, the seeds simply drop) when a dry capsule (silique) splits open. Seeds are approximately 0.3 - 0.5 mm in length (Webb et al. 1988). In the native environment *A. thaliana* seeds do not have specialised dispersal mechanisms, and are passively dispersed by wind or in soil.

1.9. Since *A. thaliana* seeds are not explosively dispersed, we do not consider that escape through ventilation systems (ie, being sucked up in airflow) is a feasible pathway of escape.

1.10. We consider that seeds being unintentionally removed from the containment facility by personnel in shoe treads, clothing or on equipment; in waste or waste water; by animals; during transit between containment facilities; or deliberate removal by unauthorised personnel are feasible pathways of escape.

Ability of pollen to escape the facility and pollinate receptive plants

1.11. *Arabidopsis thaliana* is considered to be self-pollinating, meaning pollen is transferred from an anther to the stigma of the same flower (Abbott and Gomes, 1989). The outcrossing rate for *A. thaliana* has been estimated to be less than 1% (Abbott and Gomes, 1989; Tan et al, 2005). The pollen is not known to have any specialised dispersal mechanisms to facilitate wind, insect or animal dispersal. There is no evidence to suggest that *A. thaliana* will hybridise with any other plants in New Zealand.

1.12. For GM *A. thaliana* to form a self-sustaining population outside of containment through pollen dispersal, the pollen would need to:

1. escape from containment; and
2. while viable, reach a receptive *A. thaliana* flower; and
3. fertilise the flower; and
4. the fertilised flower would need to produce viable GM seeds which must mature, disperse, and then germinate to form a GM plant; and
5. the GM plant must grow, mature, produce flowers, be fertilised etc.

1.13. The escape of *A. thaliana* pollen from a containment facility is not considered as a feasible pathway of escape.

Therefore, the feasible potential pathways for escape for *Arabidopsis thaliana* are:

- Personnel have inadequate training or supervision and mishandle new organisms resulting in release outside containment.
- Unauthorised persons enter the facility and remove seeds or whole plants.
- Seeds and whole plants are inadvertantly removed from the facility by personnel in clothing, shoe treads or on equipment.
- Seeds or whole plants escape the facility in waste water.
- Animals enter the facility and escape with seeds or whole plants.
- Seeds and whole plants accidentally being removed from containment in waste (including soil).
- Seeds and whole plants lost during transit between containment facilities.

1.14. The proposed controls (Appendix 1) and the procedures proposed to met those controls (Appendix 2) address the feasible potential pathways for escape.

Table 1 describes the containment measures proposed for *Arabidopsis thaliana* (whole plants).

Possible pathway of escape from a containment facility?	Potential measures to prevent the possible pathway of escape (these measures are similar to Guidance document Appendix 3).	Which Schedule III (Part 1) requirements are covered by these measures?	Proposed Containment Control(s) that cover this pathway (from Appendix 1)
Pathway (A) Personnel have inadequate training or supervision and mishandle new organisms resulting in release outside containment	***Management of the facility*** • Facilities will have a designated person who has overall responsibility for the containment facility. • Delegated persons may be responsible for the day-to-day operations of the facility.	**(7b)** The provision of a management plan specifying procedures for implementing controls imposed by an approval.	**1-22**

	Containment manual
	• A manual will cover:
	  ○ The management structure and areas of responsibility for the facility
	  ○ Structural and operational procedures for containment
	  ○ Qualitiative assurance measures to demonstrate effective containment
	  ○ Training of personnel in the operational procedures and frequency of refresher training
	  ○ Demonstrate number of entrances in facility
	  ○ Internal audit procedures
	  ○ External audit procedures
	  ○ The keeping of records
	• This manual will be required for facility approval by MPI under s 39 of the Biosecurity Act 1993.
		(7b) The provision of a management plan specifying procedures for implementing controls imposed by an approval.	**3**

	Training
	• All personnel will be trained on the containment practices of the organsiation relative to their responsibility.
	• Only trained personnel are permitted to work within the facility. They will:
	  ○ Be trained on the containment practices for this facility (using the operation manual).
	  ○ undergo yearly refresher courses on containment practices.
		(7a) The qualifications required of the person responsible for implementing the controls imposed by an approval.	**13, 16**

	Internal monitoring
	• The audit will:
	  ○ check that personnel are complying with the practices specified in the containment manual.
	  ○ check the physical aspects of the containment facility
	• Internal audit findings will be documented and any remedial actions taken.
		(6) Controls imposed by an approval shall specify inspection and monitoring requirements for containment facilities, including any inspection required before commencement of the development.	**18, 20**

	External monitoring • External (ie not from within the organisation being monitored) monitoring is carried out by MPI inspectors.	**(6)** Controls imposed by an approval shall specify inspection and monitoring requirements for containment facilities, including any inspection required before commencement of the development.	**19, 21, 22**
	Records Records must be maintained for auditing purposes		**4, 19**
	Contingency plans The contingency plans for the accidental spillage of new organism within the facility (for microorganisms) or by the release of the new organism outside the containment facility (breach of containment) through accident, deliberate action, natural disaster, fire, sabotage or theft or in transit between facilities. Contingency plans will be implemented if it is suspected that viable material has been removed from containment (eg, by unauthorised persons). The approval user will ensure that within 24 hours of the discovery of any breach of containment[3], the MPI Inspector responsible for supervision of the facility has received notification of the breach, and the details of any action taken by the facility since the breach occurred.	**(5a)** Controls imposed by an approval shall specify an eradication plan for escaped organisms.	**17**
Pathway (B):	***Construction of facility***	**(1f)** Requirements for	**1, 5, 6, 20, 22, 23,**

[3] Breach of containment includes: the escape of an organism(s), unauthorised entry and/or structural integrity of facility compromised.

Seeds or whole plants being intentionally removed from containment by unauthorised persons.	• Facilities are structurally sound and reasonably sealed (eg, no gaps in walls, floors).	facility construction	**24**
	Access • Doors to the facilities are kept closed except for entry and exit. • Doors in the facility are locked to restrict access to facility to authorised personnel. • Visitors (eg, tradespersons) authorised by the Operator (eg, authorised visitors) will be accompanied at all times by authorized personnel.	**1c)** Requirements to be complied with for the access of persons to the facility. **(2b)** The numbers of entrances and access to the facility **(2c)** Security requirements for the entrances and the facility	**1, 4, 5, 6, 7, 11, 12, 13**
	Signs Signs are placed at the facility entrance stating that access to the facility is restricted to authorised personnel.	**(2a)** Means of identification of all entrances to the facility	**11**
	Contingency plans	***See above***	**17**

Pathway (C): Seeds or whole plants caught in shoe treads or clothing, or on equipment or in documents and carried out of facility.	***Protective clothing*** ● Personnel wear laboratory coats and closed footwear (see below). ● Laboratory coats are only worn within the facility. ● There is a storage area for laboratory coats and other protective gear near the entrance of the facility. ● Laboratory coats are laundered on a regular basis. Laboratory coats or other clothing that maybe contaminated with new organisms are decontaminated (eg, by autoclaving or soaking in Virkon™) prior to laundering. ● To prevent plant material/plants from being accidentally removed from the containment facility in shoe treads: ○ Immature seed pods are enclosed (ie, bagged or the whole plant contained (eg, a container or growth chamber) to prevent seed dispersal. Plants are kept in trays so that should any seeds drop they will be captured within the tray. AND ○ overshoes or dedicated facility footwear are worn within the facility. OR ○ sticky mats or other mat to catch any plant material that may be walked out (eg, coir mats) are found inside facility entrances. ***Cleaning*** Measures must be put in place so that should plant material (eg, seeds) be spilled within the facility, it is not spread throughout the facility. ● The floors, walls and work benches must be smooth, easy to clean, impermeable to liquids and resistant to commonly used reagents and disinfectants. ● Areas under benches, equipment and cabinets must be accessible for cleaning. ● Dedicated wet and dry vacuum cleaners may be used. ● Benches and work surfaces are cleared at the end of each working day and disinfected.	**(1b)** Equipment and requirements for facility construction to enable the requirements for treatment and decontamination to be readily met **(1f)** Requirements for facility construction **(4a)** Requirements to prevent the contamination of work surfaces, equipment, clothing, and the facility generally	**1, 5, 6, 7, 8, 13, 14, 15, 16, 18**

	Equipment • Items are stored within the facility in a manner to minimise potential contamination from spills. • reference documents and other documents and for writing up are stored separate from lab benches. • Potentially contaminated equipment is cleaned after use. • Potentially contaminated surfaces are cleaned before maintenance or repair of equipment is conducted.	**(1b)** Equipment and requirements for facility construction to enable the requirements for treatment and decontamination to be readily met. **1f)** Requirements for facility construction **(4a)** Requirements to prevent the contamination of work surfaces, equipment, clothing, and the facility generally	**1, 15**
	Contingency plans	***See above***	**17**
Pathway (D): Seeds or whole plants washed out of facility (eg, waste water overflowing and seeping through walls or floor or running under the facility entrance doorway, waste water flowing down drains).	***Drainage*** • The floor is designed so that waste water is collected and only drains into dedicated drains. The floor, lower parts of walls and sills under doors must be constructed and sealed to ensure that liquids drain only into drains. AND • All drains empty into collecting tanks and the effluent decontaminated using autoclaving or treatment (*Note: the method chosen must render plant material non-viable*). OR Drains are fitted with mesh to filter out the viable plant material including seeds. The plant material collected must be disposed of as solid waste. OR Drains empty into a managed waste water system (eg, sewer) that must kill the plant material.	**(1a):** Requirements for treatment and decontamination to prevent escape by way of expelled air, discharge of water or liquid waste, removal of solid waste or goods, or breaches in facility boundary. **(1b)** Equipment and requirements for facility construction to enable the requirements for treatment and decontamination to be readily met **(1e)** Requirements for the disposal of any biological material. **(1f)** Requirements for facility construction **(1g)** Requirements to secure the facility and openings, including securing against failure in the event of foreseeable hazards	**1, 4, 5, 6, 18, 19, 21, 22**

	Contingency plans	***See above***	**17**
Pathway (E): Seeds or whole plants being removed from the facility by small animals (rodents) or insects.	***Construction:*** • Facilities are structurally sound and reasonably sealed (eg, no gaps in walls, floors). • Freestanding plant containment facilities have an anteroom for entry and exit. • The outer doors of the facility will be self-closing. ***Screening of drains*** Drains must be fitted with mesh to prevent insect or rodent entry. ***Screening of other openings*** Windows (if they are not sealed closed), ventilation inlets and outlets, and drains are screened with mesh to prevent rodents or insects entering the facility.	**(1f)** Requirements for facility construction **(1g)** Requirements to secure the facility and openings, including securing against failure in the event of foreseeable hazards **(3c)** Requirements to secure the facility and openings against likely unwanted organisms	**1, 4, 5, 6, 14**
	Pest control Vermin control measures (eg, a rodent control program which involves traps placed at strategic locations and checked weekly) are in place. Insect control measures (ie, sticky strips) are in place.	**(3a)** Monitoring requirements to establish the presence of other organisms **(3b)** Phytosanitary requirements	**1, 4, 5, 6, 14**
	Contingency plans	***See above***	**17**

Pathway (F): Seeds or whole plants being removed in solid waste or in soil accidentally removed from the facility.	***Disposal*** • All plant waste and soil is treated to kill any plants or heritable material before being disposed of like general rubbish. • If waste is to be killed off-site, it is securely contained within sealed containers (double containment) and then transported to the decontamination location with MPI approval. Ongoing feedback at success should be obtained for ongoing monitoring of effectiveness. Once this material is made non-viable, is disposed of like general rubbish. • Cleaners do not empty biohazardous rubbish. • Contingency plans will be put into action if it is suspected that viable material has been removed in waste. • The approval user will ensure that within 24 hours of the discovery of any breach of containment[4], the MPI Inspector responsible for supervision of the facility, has received notification of the breach, and the details of any action taken by the facility since the breach occurred.	**(1a):** Requirements for treatment and decontamination to prevent escape by way of expelled air, discharge of water or liquid waste, removal of solid waste or goods, or breaches in facility boundary. **(1e)** Requirements for the disposal of any biological material **(5a)** Controls imposed by an approval shall specify an eradication plan for escaped organisms.	**1, 4, 5, 6, 14**
Pathway (G): Seeds or whole plants escape during transit to another facility	***Movement or Transfer*** Transfers require MPI approval.	**(1d)** Procedures and requirements for transport, identification, and packaging for all biological material to and from the facility and within the facility	**8, 9, 10**
	Packaging • Viable plant material transferred to another facility (including exports) is securely double packaged and labelled. To do this: ○ the primary package (which contains the viable plant material) is sealed in a transparent container (can include plastic bags). ○ the outer package is constructed of sturdy packaging material. ○ the package's label is attached to the outer package and states that the package must only be opened within a	**(1d)** Procedures and requirements for transport, identification, and packaging for all biological material to and from the facility and within the facility	**8, 9, 10**

[4] Breach of containment includes: the escape of an organism(s), unauthorised entry and/or structural integrity of facility compromised.

	containment facility.		
	Identification and registers • GMOs will be clearly labelled. • A register to demonstrate the organism comes under a HSNO approval, to allow tracking plant/seed provenance..	**(1d)** Procedures and requirements for transport, identification, and packaging for all biological material to and from the facility and within the facility	**8, 9, 10**
	Contingency plans	***See above***	**17**

Appendix 4: Summary of submissions

1.1. We received a submission from the Ministry of Primary Industry (MPI). We did not receive any comments from the Depeatment of Conservation.

1.2. While MPI raised issues over the exact wording of controls proposed by the applicants, they support the intent of the applications. The recommended controls drafted by the EPA staff took careful consideration of MPI feedback.

1.3. Seven public submissions were received. Five supported, one opposed, and one submisson neither supported nor opposed the applications. The key points were detailed in the main advice document.

Appendix 5: Expert Feedback on Review of Guidance document

1.1 We received feedback from four recognised Arabidopsis experts who reviewed the Guidance docuiment produced from the panel of Biological Safety/ HSNO Compiance Officers.

- Dr Roger Hellens, Science Group Leader, Genomics, Plants and Food Research;
- Dr Tim Strabala, Senior Scientist and Project Leader (Molecular Forestry Programme), Scion;
- Associate Professor Joanna Putterill, University of Auckland;
- Bruce Veit, Senior Scientist, AgResearch Grasslands; and
- Dr Candice Sheldon, Scientific Regulatory Affairs Manager, CSIRO Plant Industry and Food Health and Life Science Industries Group.

1.2 The comments were used to strengthen the Guidance document (Appendix 2).

Appendix 6: Qualitative descriptors for assessment of beneficial and adverse effects

This section describes how the Agency staff and the Authority address the qualitative assessment of risks, costs and benefits. Risks and benefits are assessed by estimating the magnitude and nature of the possible effects and the likelihood of their occurrence. For each effect, the combination of these two components determines the level of the risk associated with that effect, which is a two dimensional concept. Because of lack of data, risks are often presented as singular results. In reality, they are better represented by 'families' of data which link probability with different levels of outcome (magnitude).

The magnitude of effect is described in terms of the element that might be affected. The qualitative descriptors for magnitude of effect are surrogate measures that should be used to gauge the end effect or the 'what if' element. Tables 1 and 2 contain generic descriptors for magnitude of adverse and beneficial effect. These descriptors are examples only, and their generic nature means that it may be difficult to use them in some particular circumstances. They are included here to illustrate how qualitative tables may be used to represent levels of adverse and beneficial effect.

Table 1 Magnitude of adverse effect (risks and costs)

Descriptor	Examples of descriptions - ADVERSE
Minimal	Mild reversible short term adverse health effects to individuals in highly localised area Highly localised and contained environmental impact, affecting a few (less than ten) individuals members of communities of flora or fauna, no discernible ecosystem impact Local/regional short-term adverse economic effects on small organisations (businesses, individuals), temporary job losses No social disruption
Minor	Mild reversible short term adverse health effects to identified and isolated groups Localised and contained reversible environmental impact, some local plant or animal communities temporarily damaged, no discernible ecosystem impact or species damage Regional adverse economic effects on small organisations (businesses, individuals) lasting less than six months, temporary job losses Potential social disruption (community placed on alert)
Moderate	Minor irreversible health effects to individuals and/or reversible medium term adverse health effects to larger (but surrounding) community (requiring hospitalisation) Measurable long term damage to local plant and animal communities, but no obvious spread beyond defined boundaries, medium term individual ecosystem damage, no species damage Medium term (one to five years) regional adverse economic effects with some national implications, medium term job losses Some social disruption (e.g. people delayed)
Major	Significant irreversible adverse health effects affecting individuals and requiring hospitalisation and/or reversible adverse health effects reaching beyond the immediate community

	Long term/irreversible damage to localised ecosystem but no species loss Measurable adverse effect on GDP, some long term (more than five years) job losses Social disruption to surrounding community, including some evacuations
Massive	Significant irreversible adverse health effects reaching beyond the immediate community and/or deaths Extensive irreversible ecosystem damage, including species loss Significant on-going adverse effect on GDP, long term job losses on a national basis Major social disruption with entire surrounding area evacuated and impacts on wider community

Table 2 Magnitude of beneficial effect (benefits)

Descriptor	Examples of descriptions -BENEFICIAL
Minimal	Mild short term positive health effects to individuals in highly localised area Highly localised and contained environmental impact, affecting a few (less than ten) individuals members of communities of flora or fauna, no discernible ecosystem impact Local/regional short-term beneficial economic effects on small organisations (businesses, individuals), temporary job creation No social effect
Minor	Mild short term beneficial health effects to identified and isolated groups Localised and contained beneficial environmental impact, no discernible ecosystem impact Regional beneficial economic effects on small organisations (businesses, individuals) lasting less than six months, temporary job creation Minor localised community benefit
Moderate	Minor health benefits to individuals and/or medium term health impacts on larger (but surrounding) community and health status groups Measurable benefit to localised plant and animal communities expected to pertain to medium term. Medium term (one to five years) regional beneficial economic effects with some national implications, medium term job creation Local community and some individuals beyond immediate community receive social benefit.
Major	Significant beneficial health effects to localised community and specific groups in wider community Long term benefit to localised ecosystem(s) Measurable beneficial effect on GDP, some long term (more than five years) job creation Substantial social benefit to surrounding community, and individuals in wider community.
Massive	Significant long term beneficial health effects to the wider community Long term, wide spread benefits to species and/or ecosystems Significant on-going effect beneficial on GDP, long term job creation on a national basis Major social benefit affecting wider community

The likelihood applies to the composite likelihood of the end effect, and not either to the initiating event, or any one of the intermediary events. It includes:

- the concept of an initiating event (triggering the hazard), and
- the exposure pathway that links the source (hazard) and the area of impact (public health, environment, economy, or community).

Thus, the likelihood is not the likelihood of an organism escaping, or the frequency of accidents for trucks containing hazardous substances, but the likelihood of the specified adverse effect resulting from that initiating event. It will be a combination of the likelihood of the initiating event and several intermediary likelihoods. The best way to determine the likelihood is to specify and analyse the complete pathway from source to impact.

Likelihood may be expressed as a frequency or a probability. While frequency is often expressed as a number of events within a given time period, it may also be expressed as the number of events per head of (exposed) population. As a probability, the likelihood is dimensionless and refers to the number of events of interest divided by the total number of events (range 0-1).

Table 3 Likelihood

Descriptor	Description
Highly improbable	Almost certainly not occurring but cannot be totally ruled out
Very unlikely	Considered only to occur in very unusual circumstances
Unlikely (occasional)	Could occur, but is not expected to occur under normal operating conditions.
Likely	A good chance that it may occur under normal operating conditions.
Highly likely	Almost certain, or expected to occur if all conditions met

Using the magnitude and likelihood tables a matrix representing a level of risk/benefit can be constructed.

In the example shown in Table 4, four levels of risk/benefit are allocated: A (negligible), B (low), C (medium), and D (high). These terms have been used to avoid confusion with the descriptions used for likelihood and magnitude, and to emphasise that the matrix is a tool to help decide which risks/benefits require further analysis to determine their significance in the decision making process.

For negative effects, the levels are used to show how risks can be reduced by the application of additional controls. Where the table is used for positive effects it may also be possible for controls to be applied to ensure that a particular level of benefit is achieved, but this is not a common approach. The purpose of developing the tables for both risk and benefit is so that the risks and benefits can be compared.

Table 4 Level of risk

Magnitude of effect

Likelihood	Minimal	Minor	Moderate	Major	Massive
Highly improbable	A	A	A	B	B
Very unlikely	A	A	B	B	C
Unlikely	A	B	B	C	C
Likely	B	B	C	C	D
Highly likely	B	C	C	D	D

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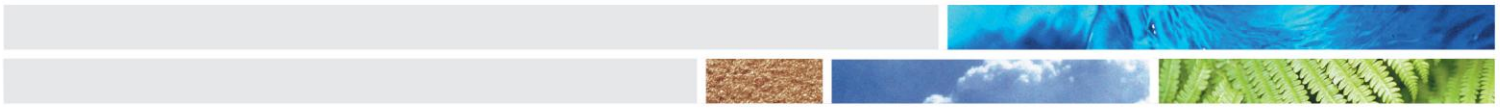

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