

**Guidelines for holding and researching non-native invertebrate biological control agents under contained conditions in England**

Under the Wildlife and Countryside Act 1981, animals that are not ordinarily resident in, and are not a regular visitor of, Great Britain are not allowed to be released into the environment in England, unless they have a Defra licence. However, these animals may be held under contained conditions without a licence. While safer than releasing these animals into a glasshouse or into the wider environment, this still presents a certain level of risk of these animals escaping into the environment. This document provides guidelines for preventing the escape of invertebrate biological control agents when held and used in research under contained conditions.

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**Containment facilities**

**Buildings**

**General design & location**

Facilities must be of suitable design and construction and be appropriately maintained in order to withstand the normal climatic conditions of the area without compromising containment. The location should also be carefully selected to prevent containment from being compromised, such as by damage from branches from overhanging trees, by flooding or proximity to publicly accessed areas where vandalism could occur. The immediate area around the facility should be maintained to good levels of hygiene by the clearing of wild or cultivated plants, and areas further away from the facility should be surveyed for plants which could act as potential hosts or reservoirs for the invertebrate biological controls. Such plants should be removed where possible and, when not possible, regular monitoring of these plants is advised. There should be appropriate security measures (guards, perimeter fencing, locked doors and appropriate signs) to prevent unauthorised entry.

**Layout**

For all containment facilities, physical isolation (including adequate separation in the same space) of the invertebrate biological controls and their prey from plants and other organisms is required. This could be achieved using secondary containment within cages or dedicated, distinct compartments within the facility. The growing of ornamental plants for decorative purposes is **not** advised within the containment facilities.

Wherever possible, the facilities in which the invertebrates are to be handled (including waste disposal) should be in relatively close proximity to each other in order to reduce the likelihood of escape during transfer, and facilities used for high risk work should, ideally, be interconnected with no requirements to take the material out of the containment facilities. Rooms where the invertebrates are handled should not have direct access to the outside.

**Entrance**

Where possible, entry to containment facilities should be via an entrance lobby with a vestibule or interlocking door system. This can be effectively achieved by not allowing the two lobby doors to be open at the same time and this is best achieved by the use of an audible/visible alarm system or electronic locking mechanism. All doors should be sealed with appropriate rubber or brush seals and should be self-closing. In some circumstances, maintaining a facility at negative pressure can be an effective method of sealing entrance doors without the need for a vestibule.

**Flooring**

Containment facilities should have permanent flooring that is well maintained and has no gaps, including any gaps between the floor and the wall (e.g. bunding).

**Drainage**

The flooring should be designed and built in a way that will reduce the risk of run-off. Facilities which are being used to contain invertebrates which are soil or water borne should have a dedicated drainage system which can either be blocked off (e.g. with the use of an appropriate filter) or connected to an appropriate quarantine treatment tank (e.g. heat treatment or chemical disinfection). In facilities where automatic watering systems are in place, the flow rates should be carefully controlled in order to avoid flooding and, wherever possible, a manual system of watering should be adopted.

**Windows and sky lights**

Where windows or skylights are present in a containment facility, these should be sealed and permanently closed e.g. screwed shut or locked (keys should **not** be left in locks), and ideally covered to prevent any movement of invertebrates towards the light.

**Pressure differentials & air filters**

Where it has been identified that passive air-borne dispersal could offer an escape route for the invertebrates, the facility should be maintained at a negative pressure with respect to both connecting facilities and the environment. This means that air is constantly being drawn into the facility and this is generally achieved by manipulating the fans responsible for both drawing in and expelling air from the facility. Air filters of appropriate design and technical specification should be in place at all points of air ingress and exhaust from the facility. The different grades of air filters available are described in Appendix II.

In general, positive pressure is **not** acceptable for containment. However, in exceptional circumstances, a positive pressure facility may be acceptable if the work exclusively involves invertebrates with no risk of airborne dispersal (e.g. nematodes). Although, where mobile vectors can transmit the organism in question, significant measures should be in place to ensure they do not enter.

**Equipment**

**Secondary containment of invertebrates within cages**

This may be necessary in certain situations e.g. when there is a need to restrict the potential spread within a facility. This is particularly applicable for mobile invertebrates. Whenever caging is considered necessary, it should be of appropriate design and construction for the material or invertebrates in question.

**Plant pots/containers**

Plants in association with invertebrates must be grown in suitable containers. Where there is the requirement to re-use containers in future experiments, they should be composed of a material capable of preventing penetration by plant roots, and which can be easily disinfected.

**Systems of work**

**Security**

As people entering and leaving facilities may offer an efficient method for disseminating invertebrates, access should be restricted to authorised personnel only. This is most easily achieved by having lockable outer doors; the use of key-pads, electronic swipe cards, Yale type locks or padlocks should be encouraged. Mortice type locks are not appropriate.

**Hygiene**

Protective clothing, such as laboratory coats, overalls, gloves and footwear must be worn as appropriate. These should be clearly marked and left within the facility on exit, preferably in a lobby area with hand-washing facilities and a footbath (where appropriate). Used/contaminated clothing should be decontaminated prior to laundering, which could involve autoclaving (121°C [15psi] for 15 minutes) or freezing at -15°C for a minimum of 72 hours.

**Pest control**

There should be effective control of glasshouse pests which could disseminate the invertebrates, either directly as vectors or by the removal of contaminated material. This could be achieved by screening all possible points of entry and adopting additional measures where appropriate, such as below ground barriers to exclude rabbits. Efficient invertebrate vector control can be achieved by: i) adopting the limited use of mechanical/manual ventilation systems, ii) screening of all air inlets and exhausts to a suitable size for the vectors concerned, iii) using a vestibule/lobby arrangement to enter the facility, iv) fitting brushes or rubber strips around all doors and v) using regular, appropriate chemical control regimes and traps (e.g. yellow sticky traps for aerial insect vectors and sticky foot traps or footbaths containing disinfectants at the exits).

**Containment of invertebrates**

It is likely that the containment of invertebrates in association with plants will only be permitted in dedicated growth cabinets/rooms or insectary facilities. These should involve the minimum number of plants, be of short duration, and preferably be undertaken when the environmental conditions outside the facility are less likely to permit the survival of the invertebrate. The construction of a specialist insectary facility containing growth cabinets in which plants can be grown is encouraged, with the application of temperature and light gradients providing additional barriers to invertebrate movement.

Plants may be grown and prey may be reared separately outside of containment facilities, providing they are not associated with the invertebrate biological control agents. When they are used with the invertebrate biological control agents, they should be treated as a risk of spreading the invertebrate biological control agents and disposed of appropriately (as described later in the document).

**Transfer of material between facilities**

The procedures involving the transfer of invertebrates between facilities should limit the risk of escape of the material. Transfer of invertebrates between facilities on site must be kept to a minimum, but where transfer is unavoidable invertebrates should be transported within three layers of containment. This may involve the use of sealed bags and/or closed, sealed, shatterproof containers as appropriate.

**Labelling**

Rooms, storage units and containers where the invertebrates are being used or held should be adequately labelled.

**Staff**

Staff should be adequately trained and aware of the conditions required to contain the invertebrates.

**Record keeping**

Records should be kept of the following:

* Site plan, showing where the invertebrates are kept and worked on
* Consignments of the invertebrates being imported or moved to and from the facilities
* Work activities carried out using the invertebrates
* Staff and training records
* Visitor records

**Contingency planning**

Procedures should be in place in the event of a breach of containment conditions. If the invertebrates escape from the containment facilities, Defra should be notified ([non-nativebiocontrol.licensing@defra.gsi.gov.uk](mailto:non-nativebiocontrol.licensing@defra.gsi.gov.uk)).

**Waste**

All contaminated waste, including packaging material, pots, soil and plants associated with the invertebrates must be inactivated by validated means prior to disposal. Autoclaving of material will provide the best assurance. Where incinerators are available on site, these may be used. Wherever possible, autoclaves should be available for use and a temperature of 121°C (and a pressure of 15 psi) must be maintained in the centre of the load for a minimum of 15 minutes for the treatment of invertebrates, and 30 minutes if soil and plant material is present. Wherever possible, autoclaves must be located as close to the working area as possible, with any movement outside of the facility only permissible in contained transfer carriers (in three layers of containment). Freezing of the invertebrates and contaminated waste (not including soil and plants) at -15°C for a minimum of 72 hours, is also acceptable. See Appendix I for a more detailed overview of waste disposal options.

Table 1 provides a summary of the potential routes of escape for quarantine organisms. It also attempts to summarise both the physical containment facilities and procedural measures that are advised to reduce the likelihood of escape via these routes. It is not intended to be an exhaustive summary and there may be other measures which should be applied.

**Table 1**

|  |  |  |
| --- | --- | --- |
| **Route of escape** | **Physical containment** | **Procedural containment** |
| Natural / deliberate damage to facility and dissemination of plants with associated invertebrates or the invertebrates themselves | 1. facility of suitable design, construction & location to withstand normal climatic conditions of the area 2. facility of suitable construction & location to withstand possible vandalism | 1. facility appropriately maintained 2. good security 3. restricted access 4. approved contingency/emergency plans in place to cope with damage |
| Deliberate human removal of invertebrates (other than for work purposes) | 1. facility locked and/or alarmed 2. appropriate signs | 1. good security 2. restricted access 3. Standard Operating Procedures (SOPs) |
| Movement of invertebrates between facilities | 1. geographically interconnected/close proximity of facilities to limit movement 2. appropriate containers for transfer and storage 3. dedicated facilities/work areas not used for growing other plants 4. secondary containment | 1. restricted access 2. good staff training and adherence to SOPs 3. good hygiene & hygiene facilities (e.g. protective clothing, handwashing, sticky mats/footbaths on exit) 4. movement within three layers of containment one of which should be shatterproof 5. freezing of protective clothing and associated material where appropriate |
| Dissemination of plants with associated invertebrates or of invertebrates themselves by vermin (e.g. rodents & birds) | 1. all openings appropriately sealed/screened 2. self-closing, appropriately sealed double doors 3. secondary containment of plants where appropriate | 1. clean and tidy facilities 2. appropriate pest control regimes in operation 3. spatial isolation from potential host plants, including monitoring the area surrounding the facility |
| Dissemination of invertebrates by non-quarantine invertebrate or fungal vectors | 1. all openings appropriately sealed/screened 2. facility under negative pressure 3. filtered air exhausts 4. secondary containment of plants within cages of suitable design & construction 5. self-closing, appropriately sealed double doors 6. UV light, sticky traps & sticky benching 7. temperature/light gradients 8. pots in trays/saucers 9. controlled drainage water (blocked drains, trap, sump/quarantine treatment tank) | 1. clean and tidy facilities 2. appropriate pest control regimes in operation 3. good hygiene & hygiene facilities (e.g. protective clothing, handwashing, sticky mats/footbaths on exit) 4. freezing of protective clothing and associated material where appropriate 5. appropriate sticky mats/footbaths on exit 6. spatial isolation from potential host plants, including monitoring area surrounding facility |
| Dissemination of nematodes by plant seeds | 1. all openings appropriately sealed/screened 2. exhaust air filtered to appropriate level 3. facility under negative pressure if necessary 4. secondary containment of plants within cages of suitable design & construction 5. reduced air movements and vortices 6. self-closing, appropriately sealed double doors with sills 7. controlled drainage water (blocked drains, trap, sump/quarantine treatment tank) | 1. prevent seed set 2. bag flowers 3. limit numbers of plants 4. spatial isolation from suitable seed germination sites (e.g. treat area around facility) 5. seasonal isolation from suitable seed germination periods 6. monitor area surrounding facility for seedlings |
| Dissemination of invertebrates via water/soil -borne routes | 1. pots standing on trays/saucers 2. controlled drainage water (blocked drains, trap, sump/quarantine treatment tank) 3. self-closing, appropriately sealed double doors with sills | 1. clean and tidy facilities 2. good hygiene (e.g. protective clothing, overshoes, footbaths etc.) 3. controlled/minimal watering (excess lost by evaporation & flooding prevented) 4. avoid soil aerosols 5. spatial isolation from potential host plants, including monitoring the area surrounding the facility |
| Dissemination of invertebrates by unsatisfactory waste inactivation | 1. appropriate equipment in place (e.g. autoclave, incinerator) 2. appropriate containers for transfer and destruction 3. appropriate disposal post-inactivation (e.g. deep burial) | 1. personnel training 2. regular, appropriate equipment maintenance 3. appropriate chemical disinfectants for spillage/disposal 4. appropriate clearance of soil sumps/quarantine treatment tanks |

**APPENDIX I**

**Destruction/sterilisation options**

Sterilisation is strictly defined as the non-selective inactivation of all organisms in a given substrate.

The sterilisation treatment should be determined in accordance with the following considerations:

i) the type and nature of the waste material

ii) the efficiency of the treatment method

iii) the operating conditions of the treatment method.

**Examples of different types of appropriate sterilisation/destruction methods**

**i)** **Incineration (on site) - Oxidation to ashes at 1000°C.**

Note: Soil may be sent off site for incineration, provided the following conditions can be met:

* The contractor undertaking the incineration must have an appropriate (waste disposal) licence from the local authority.
* The soil should be packaged appropriately (within 3 layers) before transport and should be transported directly to the incinerator.
* The soil should be incinerated immediately upon arrival at the incinerator (within 48 hours) – it should not be stored for incineration at a later date.

However, off-site incineration should only be permissible when the volume of soil to be disposed of makes on-site treatment impractical.

**ii)** **Moist heat - Autoclaving at 121°C (15 psi) for a minimum of 15 minutes; 30 minutes for soil/plant material.**

This is the best method of sterilisation and should be used where possible.

Ensure autoclavable bags are open to allow steam to penetrate, and add water to completely dry material. Temperature probes or temperature strip indicators should be used to ensure sterilisation conditions have been achieved. Validation should periodically be carried out to ensure the invertebrate is being killed by the autoclave conditions, and autoclaves should be serviced regularly. Factors such as load size and type of container used may also need to be taken into consideration as this can affect steam/heat penetration and may require longer times.

**iii) Dry Heat - Oven at 180°C for 1 hour, 160°C for 2 hours**

Suitable only for glassware and equipment unaffected by heat.

**iv) Dry Heat - Furnace at 400-450°C for 4 hours (or until reduced to ash)**

Suitable for smaller quantities of soil or quantities of seed etc.

**v) Freezing - Invertebrates, hold at ≤ -15°C for 72 hours before disposal**

**vi) Chemical - Treatment of liquid waste, or formaldehyde, hydrogen peroxide, chlorine dioxide or ethylene oxide as gaseous fumigants for disinfection of labs and safety cabinets/fume hoods.**

Note: formaldehyde is a possible carcinogen and has been banned in some EU countries and an EU-wide ban may occur in the future.

**vii) Boiling - 100°C for a minimum of 30 minutes.**

**viii) Aerobic digestion/composting**

The breakdown of plant and organic material during composting is mostly achieved by saprophytic bacteria, but is also aided by fungi. Mesophilic bacteria are responsible for decomposition in the first and final stages of composting. The middle phase, where temperatures can increase to between 45 and 70°C, is achieved by thermophilic bacteria. However, these temperatures may only be achieved in the centre of the heap, meaning there can be a lot of heterogeneity within the compost. These temperatures can be achieved for just a few days or up to several months. This procedure should be shown to be effective against the invertebrate that is being worked on and composting should be sufficiently contained to prevent any escape (particularly with respect to airborne invertebrates).

**ix) Destructive analysis**

The process of destructive analysis is considered to be completed for soil and other organic material by the addition of strong acids or alkalis, especially with heat as in a digestion process, or as in extraction processes using industrial solvents (50-60 types) ranging from ethanol to trichloromethane.

**Alternative methods**

Alternative methods of destruction may be used if accompanied by supporting data (normally required to be published in a peer reviewed journal) showing that the treatment is both a) effective against the target organism/s and b) effective in the situation it is to be used in.

**Disposal of waste**

The options available for the disposal of waste and waste effluent following sterilisation or destruction are primarily:

1. authorised landfill or similar disposal sites,
2. discharge to the sewer system – Restricted. It is advised to check the material safety data sheet (MSDS) for chemical disinfectants, which will contain safety (S) and risk (R) phrases for each of the chemicals in the product and should give information on disposal of the product. Guidelines for disposal to sewers is set out and enforced by local water authorities.

The selection of the appropriate option should be based on the sterilisation treatment used, the nature of the waste itself, environmental effects, reliability and cost.

**APPENDIX II**

**Air filters**

Facilities in which aerially dispersed invertebrates are held should have appropriate filters in place at all air vents in order to prevent escape.

High Efficiency Particulate Absorption (HEPA) filters offer the highest level of containment and should be used whenever possible.

Standard air filters are categorised into one of 2 groups, with each group having a number of different subclasses:-

1. Group G[[1]](#footnote-1) (coarse dust filters), classes: G1-G4 (EU[[2]](#footnote-2) 1-4)

1. Group F10 (fine dust filters), classes: F5-F9 (EU11 5-9)

The filter sizes and efficiency is listed in the table below.

You may come across filters with just MERV standards listed. These are the American standards and are included in table 2.

G1 filters represent the most basic standard filter with F9 filters representing the best filtering efficiency below the HEPA standard.

The higher the filtering capacity, the more resistance there will be to air flow. This means that the higher specification filters (e.g. HEPA) tend to be restricted to more specialised applications involving smaller air volumes (e.g. microbiological safety cabinets) and will often be inappropriate for handling large volumes of air.

From a containment perspective, the filters in place at the air exhaust from any containment facility need to be appropriate for preventing the escape of the smallest airborne stage of the invertebrate. It is important that filters are sufficient to prevent the organisms from forcing their way through the weave of the filter.

G3 filters are rated as being 98% efficient at trapping airborne particles 10 μm in diameter. However, G3 filters are not generally suitable for containing invertebrates (e.g. aphids) which could force themselves through the weave of the filter.

G4 filters on the other hand are made from pleated panels and are rated as being over 90% effective at arresting particles of 4μm in diameter as well as being much more effective at containing invertebrates.

The table below can be used to work out the minimal filter grade that will be effective for the work being carried out.

**Table 2.** UK standard filter grades.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **EU standards** | **US standards** | **Particle size range (µm)** | **Arrestance of synthetic dust** | **Arrestance of 0.4 µm particles** |
| G1 | MERV 1-4 | >10 | <65% |  |
| G2 | MERV 1-4 | >10 | <80% |  |
| G3 | MERV 5 | 3-10 | <90% |  |
| G4 | MERV 6 | 3-10 | >90% |  |
| F5 (M5) | MERV 9-10 | 1-3 |  | <60% |
| F6 (M6) | MERV 11-13 | 1-3 |  | <80% |
| F7 | MERV 13-14 | 0.3-1 |  | <90% |
| F8 | MERV14-15 | 0.3-1 |  | <95% |
| F9 | MERV 16 | 0.3-1 |  | >95% |
| HEPA | MERV 16 | 0.3-1 |  | >98% |

The following website also gives useful information about filters: <http://www.berriman-usa.com/tutorial_2_air_purifiers.htm>

**APPENDIX III**

**General requirements when importing, moving or exporting material**

**Packaging**

All material moved should be contained in three layers of packaging, at least one of which should be both escape and shatter proof.

**Transit**

Biocontrol invertebrate material should be conveyed **directly** from the place of landing, or from the contained facility in England and Wales providing the material, to the other containment facilities.

A licence must be applied for when importing any non-native animal into the UK. Guidance on importing non-native animals can be found here: <https://www.gov.uk/guidance/importing-non-native-animals>.

**APPENDIX IV**

**Relevant links**

Plant Health controls:

<https://www.gov.uk/guidance/plant-health-controls>

Animal Health controls:

<https://www.gov.uk/government/collections/guidance-on-importing-and-exporting-live-animals-or-animal-products#non-native-animals>

The Genetically Modified Organisms (Contained Use) Regulations 2014:

<http://www.hse.gov.uk/pubns/books/l29.htm>

1. UK classification system [↑](#footnote-ref-1)
2. EU classification system [↑](#footnote-ref-2)