Biosafety guidelines for contained use activities with arthropods

This document is intended to serve as guidance for biosafety officers and users and to provide information on the correct implementation of containment criteria and other protective measures for activities with arthropods.

This document is also used by the Biosafety and Biotechnology Department to provide advice to the competent authorities on the contained use of genetically modified and/or pathogenic organisms.

This document may be updated in the light of experience acquired and any comments made by users. Comments can be sent to the following address : contained.use@sciensano.be

Content

Introduction

In Belgium, activities with arthropods in a facility such as a laboratory, animal facility or greenhouse fall within the scope of the regional legislation on contained use (CU) of genetically modified (GM) organisms and/or pathogens when these activities are intended to manipulate arthropods that are genetically modified or have a quarantine status and/or are carrying pathogenic micro-organisms (genetically modified or not) [1]. Therefore, maintaining, rearing and/or manipulating such arthropods have to be done in accordance with the appropriate containment measures and work and waste management practices proportionate to the level of risk of the activity. The aim of this containment is to avoid a release of these organisms into the environment as this may lead to risks for humans, animals or plants, to environmental disturbances and economical costs.

Because arthropods have specific characteristics such as the small size, the way they move (whether or not they can fly, swim, climb, jump or crawl) and the changing forms during life stages (eggs, larvae, nymphs, adults), this guideline aims at providing further details and practical solutions to users to implement adequately the containment level corresponding to the level of risk of their activity with arthropods in accordance to regional CU legislations. It has been drafted on the basis of existing guidelines [2-7], the experience gained in risk assessment and management of CU activities notified by users to the SBB in Belgium and inputs from experts in the field. It should be noted that so far (2023), most of these activities involve insects such as mosquitoes, bees, moths, flies or fleas as well as arachnids such as mites and ticks. In case of activities involving other classes or other species of arthropods, it is recommended to check whether the specified containment measures described in the present document are still appropriate.

Risk classification and management of CU activities with arthropods

In the framework of a CU notification and authorization request, an in-depth risk assessment of the activity involving arthropods is required. Specific elements to consider for the risk assessment of arthropods are briefly mentioned below.

- Genetically modified arthropods

The risk evaluation of GM arthropods should identify adverse effects related to the genetic modification and assess the extent to which it may alter the characteristics of the resulting organism (as compared with the non-GM organism) in a way that is potentially harmful to humans, animals and plants in particular but not limited to :
- the capacity to transmit the genetic modification to another permissive host or breeding partner;
- the increase in blood-feeding needs compared to the wild-type species;
- the decreased susceptibility to control or surveillance measures (e.g. insecticides);
- the alteration of the life-history traits such as lifespan and life cycle, viability, fertility rate, sex ratio;
- the alteration in allergenicity, toxicity;
- in the case of a disease vector arthropod, the increase in vector capacity or the acquisition of vector competence for additional pathogenic organisms.

- Vectors of pathogens

In the wild or in the lab, arthropods can be indirectly involved in diseases by carrying and transmitting pathogenic, quarantine micro-organisms or GMMs. If the arthropod itself does not pose any risks, the risk assessment should consider primarily the risks of the carried micro-organism for the human health or the environment and should consider the severity of the disease in humans, animals or plants, the availability of a treatment or prophylaxis against the disease and the economic consequences in case of infection of livestock or plant cultures. In the case of pathogens, reference lists have been developed in Belgium in which risks of a number of pathogens for human and animal health and for plants have been classified[1].
In addition to the specific risks of the carried pathogen, the capacity of the arthropod to contribute to the spread and transmission of the pathogen to humans, animals or plants should also be considered, in particular:

- its host spectrum and prevalence of these hosts in the local environment (animal or plant);
- its survival, persistence and reproduction capacities outside the containment and in the local environment;
- its potential ability to carry and transmit other pathogenic micro-organisms to humans, animals or plants.

- Quarantine arthropods

Quarantine organisms form a special group requiring special containment. Quarantine organisms are defined in the European Regulation (EU) 2016/2031 [8] as organisms harmful for plants and plant products “whose entry, establishment and spread would have an unacceptable economic, environmental or social impact on the territory”. Quarantine organisms, including arthropods, are included in lists established by the European Commission[2] [9]. In Belgium the implementation of Regulation (EU) 2016/2031 is under the responsibility of the Federal Agency for the Safety of the

[1] Tools - Belgian classification for micro-organisms based on their biological risks | Belgian Biosafety Server (biosecurite.be)

[2] The list of European Union (EU) quarantine pests and protected zone quarantine pests is set out in implementing regulation (EU) 2019/2072. The list of EU priority quarantine pests is set out in Delegated Regulation (EU) 2019/1702. There are also quarantine pests for which the European Commission has promulgated emergency measures via implementing regulations and which are not listed in Regulation (EU) 2019/2072.

Food Chain (FASFC) [10]. Because the list published by the FASFC is not always up-to-date to reflect the rapid changing situation on the field, it is recommended to consider also the most recent lists published by EPPO (European and Mediterranean Plant Protection Organization) to carry out the risk assessment [11].
In Belgium, handling quarantine arthropods in a contained facility requires an authorization from both the FASFC and the regional CU authorities.

Containment levels

The CU legislation foresees different containment levels (CL)[3] for a laboratory (L), an animal facility (A) or a greenhouse/growth chamber (G) corresponding to the level of risk of the contained use activity. When arthropods are involved in the activity, generally the containment levels should be applied as follows:

- CL1 to activities of no or negligible risk for human health and the environment. Such activities could involve GM arthropods or arthropods (GM or not) inoculated with non-pathogenic GM micro-organisms.
- CL2 to activities of low to moderate human health and/or environmental risk involving the use of arthropods (GM or not) inoculated with a pathogen (GM or not) of minimum biological class of risk 2.
- CL2-Q to activities involving the use of quarantine arthropods or arthropods (GM or not) inoculated with a quarantine micro-organism.
- CL3 to activities of high human health and/or environmental risk involving the use of arthropods (GM or not) inoculated with pathogens (GM or not) of minimum biological class of risk 3.

It is however important to note that the determination of a containment level for a specific activity is a case-by-case exercise. In some cases, the specific characteristics of an activity could justify the adoption of an adapted level of containment in relation to the general rules mentioned above.

[3] There is no containment level 4 in Belgium until now.

Specific measures regarding the containment of activities involving arthropods

The containment measures described in the CU regional legislation provide a general framework for the containment level necessary to protect the human health and the environment against potential risks arising from CU activities involving GMOs and/or pathogens. Given the particular characteristics of arthropods, it is necessary to specify some of these measures at the level of design, technical specifications of the containment facilities, safety equipment and work practices, including waste practices. Focus is given in prevention of arthropods escape from the containment as this is a major specific risk when working with such organisms. The following tables list the containment measures for the different levels of risk in a laboratory (L), an animal house (A) in case of housing or rearing arthropods or a greenhouse (G) if arthropods are manipulated/housed or reared in combination with plant cultivations. Specification of a measure is accompanied by a note explaining the function of that particular measure and sometimes by practical examples in the context of an activity with arthropods.

The specifications are presented in the tables in the same way as in the CU regional legislation and the following terms are used :

- ”Not required” means a measure that is not mandatory.
- ”Optional” means a measure to be applied on a case-by-case basis depending on the risk assessment.
- ”Recommended” means a measure to be applied as a general rule unless safety to human health and the environment is not compromised.
- ”Required” means a measure that is mandatory. However, alternative measures fulfilling the same level of safety can be proposed by the user.

It is important to note that the specifications and explanatory notes mentioned in the tables are generic and cannot cover all particular cases. As foreseen by the CU regional legislation, they should not preclude the adoption, after joint assessment with the SBB, of alternative measures which guarantee at least equivalent effectiveness and safety. In some cases, users may, with the agreement of the competent authority and the SBB, not apply a specification relating to a particular containment level or combine specifications given for two different levels.

The application of containment measures or a set of measures adapted to activities with arthropods should also take into account the intrinsic characteristics of the handled arthropod (e.g. resistance to freezing, mobility, diet, …). These may change with the life cycle, therefore requiring different containment measures at different stages. An in-depth knowledge of the manipulated arthropod is essential to select and implement effective containment measures for each of the life stages.

Laboratories (L) for handling arthropods

Notes:

The containment levels for laboratories are defined as follows:
L1 for containment level (CL) 1 activities involving handling genetically modified (GM) arthropods or arthropods (GM or not) inoculated with non-pathogenic GM micro-organisms.
L2 for CL2 activities involving handling arthropods (GM or not) inoculated with a pathogen (GM or not) of minimum class of risk 2.
L2-Q for CL2 activities involving handling quarantine (Q) arthropods or arthropods (GM or not) inoculated with a Q organism.
L3 for CL3 activities involving handling GM arthropods or wt arthropods inoculated with a pathogen (GM or not) of minimum class of risk 3.

Design and technical specifications

Specifications		L1	L2	L2-Q	L3
1.	**Laboratory suite: separated from other activity areas**	Not required	Not required	Not required	Required
2.	**Access via airlock**	Not required	Optional *	Optional *	Required

In the framework of activities with arthropods, an airlock is always useful whatever the level of containment. However, an airlock and doors with an interlock system are mandatory in L3.
* In L2-Q, the airlock is required when flying quarantine arthropods or flying arthropods infected with quarantine micro-organisms are manipulated.
Although the airlock is not required in L2, when working in this containment level with flying arthropods or arthropods deliberately infected with pathogens of class of risk 3, the airlock is recommended.

Separate airlocks for personnel and equipment can be foreseen as determined by risk assessment.

Comment :
When working with arthropods, the airlock has the following advantages :

- *it acts as a physical barrier to potentially escaped arthropods. Additionally, traps can be installed in it to reinforce prevention of escapees into the environment;*
- *it facilitates the possibility for workers to check for any escapees hanging on their clothes and on other personal protective equipment (PPE) before final exit. The use of mirrors allows visual inspection of large enough arthropods on clothes or PPE;*
- *where pertinent (cold sensitive arthropods), it can be permanently set at a controlled temperature at which arthropods cannot survive or it can be*

cooled periodically to such temperatures, especially in the event of a known/suspected escape. Alternatively or in addition to the cooled airlock, corridors may also be cooled.

- *An alternative to a permanently built airlock consists in placing a meshed space at the entrance, at least if enough space is available.*

3.	**Lockable access door**	Not required	Required	Required	Required
4.	**Self-closing access door**	Not required	Optional	Optional	Required

The access doors to the laboratory are equipped with a self-closing system when they directly give access to a public area. This system contributes also in preventing escapes.
Doors should close quickly.

5.	**Sealed windows**	Not required	Not required, but must be closed during experimentation	Required*	Required

A screen or mosquito net on windows that can be opened (L1, L2) are placed when determined by the risk assessment. Where nets are used, they should be of an appropriate sized mesh to stop the involved arthropods and robust enough to withstand airflows.

* Regional legislation on contained use stipulates that in L2-Q, windows must be closed during experiments. However, in the case of contained uses with Q arthropods or arthropods infected with Q micro-organisms, windows must be permanently sealed.

6.	**Laboratory: sealable for fumigation**	Not required	Not required	Not required	Required
7.	**Furniture designed to facilitate the insect and rodent control programme**	Not required	Recommended	Recommended	Required

This measure allows the control of insect and rodent entry into the lab, but also the escape of the handled arthropods.
The amount of furniture and other equipment is as limited as possible and where present, contrasting in colour to facilitate detection of arthropods. Equipment (for storage) such as cupboards, incubators and fridges/freezers are mobile to allow easy detection and cleaning.

8.	**An observation window or alternative so that occupants can be seen**	Not required	Optional	Optional	Recommended

An observation window or equivalent (e.g. a camera) allows to see who is in the room as well as to assess the general situation in the laboratory. This is

required if there is a risk of incident by opening the door.

Point of attention :
Light cycle is sometimes necessary to apply for adequate (temporary) maintenance of arthropods in the lab. A device (a mobile shield for instance) on the windows to hide the outside light can be foreseen. This measure is also useful to prevent light attraction of flying arthropods.

9.	**Access for staff to washing and decontamination facilities**	Required (sink)	Required (sink)	Required (sink)	Required (sink in airlock or near the exit)

In L2-Q and L3, seal drains (sink, floor, shower) with an adapted mesh to prevent escape of arthropods. The drain of the sink should be covered with an adequate filter or fine mesh to avoid the escape of any eggs or larvae.
If required by risk assessment and as a last resort (in case of accidental release), suitable insecticides or pesticides are poured in siphons and water traps.

10.	**Non-hand operated sink**	Not required	Optional	Recommended	Required
11.	**Coat hangers or lockers for protective clothing** In airlock if available	Recommended	Required	Required	Required
12.	**Fluid supply lines are fitted with backflow prevention devices**	Not required	Not required	Not required	Recommended
13.	**Surfaces resistant to water, acids, alkalis, solvents, disinfectants and decontamination agents, and easy to clean** In addition : - resistant to insecticides/pesticides - light coloured - smooth and seamless	Required for benches Required Required Not required	Required for benches Required Required Not required	Required for benches Required Required Required	Required for benches and floor Required Required Required

Surfaces including those of benches, furniture, walls, floor, ceiling, are contrasting in colour to facilitate detection of escaped arthropods. In L3 and L2-Q, surfaces are smooth and seamless with no cracks or crevices, as these provide hiding places for escaped arthropods.

14.	**Autonomous electric system**	Not required	Not required	Not required	Required

15.	**Fire detection and alarm system**	Not required	Not required	Not required	Required
16.	**System to communicate with outside zone**	Not required	Not required	Optional	Required
17 – 18	**<u>Ventilation</u>** **Supply and exhaust air ducts independent from those in adjacent rooms**	Not required	Not required	Not required	Recommended
19.	**Supply and exhaust air ducts interconnected to prevent accidental overpressure**	Not required	Not required	Required	Required
20.	**Supply and exhaust air systems that can be closed by means of valves**	Not required	Not required	Optional	Required
21.	**Negative pressure relative to the pressure of the immediate environment**	Not required	Not required	Required in manipulation zone (e.g. BSC)	Required (with control and alarm systems)
22.	**Extract and input air from the laboratory should be HEPA filtered**	Not required	Not required	Required in manipulation zone at extraction	Required at extraction
23.	**System to change filters safely**	NA	NA	Required	Required
24.	**Filtered air reusable**	NA	NA	Optional	Optional
25.	**Specific measures to adequately ventilate the area**	Optional	Optional	Recommended	Required

Additional measures specific to arthropods aiming to control possible "hot spots" for arthropod escape:				
Screened and/or sealed :				
grids, holes, gaps, key holes…	Not required	Optional	Required	Required
air inlets/outlets	Not required	Optional	Required	Required
pipework (drainage, sink…)	Not required	Optional	Required	Required
electrical service conduits (socket outlets, lighting…)	Not required	Optional	Required	Required
Low ceiling height	Not required	Optional	Required	Required

To prevent escapees in L3 and L2-Q and in L2 when determined by risk assessment (e.g. arthropods infected with a pathogen of risk class 3), screen or seal any hot spots for arthropod escape (e.g. grids, holes, gaps…) in the facility. Where screens are used, they should be of an appropriate sized mesh to stop the involved arthropods and robust enough to withstand airflows.
The entrance door is tight-fitting and when closed, all free-spaces or crevices are sealed or covered using for instance magnetic seal strips, brush barriers, flexible flanges or equivalent. Key holes on doors are avoided or adequately sealed.
Air inlets/extracts, all pipe work (e.g. drainage, including in growth chamber, sink) and electrical service conduits are sealed to prevent escapees. Ceiling mounted lighting should be flushed with the ceiling and ideally accessible from above to avoid breaching containment.

Having low ceiling heights makes easier the detection of escaped arthropods as well as their recapture. Recovered arthropods should be disposed of following procedures of infectious waste management.

Points of attention:

- *Achieve regularly an in-depth inspection of the physical containment (e.g. walls, screens, seals, mounted lights). It is also recommended to check "hot spots for escape" weekly or even daily. The frequency at which hot spots must be checked is evaluated on a case-by-case basis. Replacement must be foreseen where necessary.*
- *These measures will also prevent entry of arthropods and other unwanted animals into the room.*

Safety Equipment

Specifications		L1	L2	L2-Q	L3
26.	**Biosafety cabinet**	Not required	Optional	Optional	Required

The use of a biosafety cabinet (BSC) may be difficult with small arthropods that cannot withstand the strong airflow within the cabinet. In this case a BSC is only used to prepare the infectious material that will be inoculated to the arthropods. Then, if a primary containment is necessary during infection and handling of infected arthropods, a glovebox (without laminar airflow) can be used to isolate and allow safe manipulations without risk of escape and contamination. Immobilization (physical, cold or via anaesthesia) can be applied as an alternative. If during handling exposure cannot be excluded or if adequate immobilization cannot be foreseen, a biosafety cabinet or an (HEPA filtered) isolator is respectively required. An appropriately sized glovebox can be used inside the BSC to protect arthropods from BSC airflow.
An insecticide, pesticide or a CO_2 gas bottle is foreseen inside the device in the case of an emergency.

27.	**Autoclave**	On site	In the building	In the lab or annex room *	In the lab or annex room *

* with validated procedures allowing the safe transfer of the biological material to an autoclave outside the laboratory and offering an equivalent level of protection.

28.	**Double-ended autoclave**	Not required	Not required	Not required	Optional
29.	**Centrifuge <u>in the lab</u>**	Not required	Not required if tubes are sealed	Not required if tubes are sealed	Required
30.	**Vacuum generating system with HEPA filter**	Not required	Not required	Optional	Recommended
<u>Additional safety equipment specific to arthropods</u>					
Air curtain at entrance door of the lab		Not required	Optional	Optional	Optional

An air curtain (or equivalent) creates an impenetrable screen of air for flying or light arthropods [12].

<u>Work practices and waste management</u>

Specifications		L1	L2	L2-Q	L3
31.	**Restricted access** Controlled access	Recommended Not required	Required Optional	Required Optional	Required Required

In L3, a controlled access system such as the use of an electronic access card for authorized and trained staff is mandatory. In L2 and L2-Q, it may be

mandatory when specific training for manipulations of arthropods with specific risks is required.

32.	**Indications on the door:** a: Biohazard sign b: contact details of the person in charge c: containment level d: nature of the biological risk e: authorized access persons f: access criteria (e.g. specific training)	b, c	a, b, c, d*	a, b, c, d*	a, b, c, d, e, f

* The nature of the biological risk should be indicated on the entrance door of L2 and L2-Q due to the particular features of arthropods

33.	**Laboratory to contain its own equipment**	Not required	Not required	Not required	Required
	Reduce work flow (material, personnel and arthropods)	Not required	Minimized	Minimized	Minimized

When working with arthropods, work flows of material, staff and arthropods should be minimized by concentrating all activities with these organisms (microscopy, photography, crossing, infection, feeding…) in one room or dedicated area. This measure implies indirectly that this room or area contains its own equipment. Flow of materials and organisms should be limited to one way for activities in L3.

34 - 38	**Personal protection equipment (PPE) :**				
	Coat	Required	Required	Required	Required
	Coat decontaminated before exit from the area	Not required	Not required	Optional	Recommended
	Gloves	Not required	Optional	Optional	Required
	Respiratory protection	Not required	Not required	Not required	Optional
	Face protection	Not required	optional	Not required	Optional
	PPE specific to the activity and/or room	Not required	Optional	Required	Required

Wearing a lab coat is mandatory in all containment levels. Other personal protection equipment (PPE) are selected taking into account the risk level of the activity, the mode of transmission of the pathogens or GMMs infecting the arthropods (prick, contact, inhalation…) and the physical barriers already in place in the facility.

PPE can prevent unintentional release of an arthropod by protecting the hair, the hands, by covering shoes… or by preventing access to any hiding place. For

this reason, in L2-Q and L3, PPE should be specific to the laboratory.

Procedures for staff are in place to describe the removal and treatment of PPE.

Points of attention:

- *PPE is preferably contrasting of colour for easy detection of arthropods*.
- *As PPE is a possible escape route for arthropods that may linger or hide in it, their systematic check before removal is performed in all containment levels except L1, with or without the use of mirrors, preferably in the airlock if available.*
- *A hand-size vacuum can be foreseen (in the airlock if available) to remove possible arthropods on PPE and on area's that were not protected by PPE. Recovered organisms are disposed of following procedures of infectious waste management.*
- *It is useful to wear appropriate PPE that prevents escape such as lab coats without pockets, with zips, cuffed sleeves and trouser legs, etc.*
- *To further reduce this risk, it may be considered to freeze the PPE (-15°C or lower depending on the arthropod resistance to cold) while awaiting reuse.*
- *In L3 and where determined by risk assessment in L2-Q, lab coats should be decontaminated before leaving the lab.*

39.	**Physical containment of micro-organisms or viable organisms (closed system)**	Recommended (one-layer containment)	Required (one-layer containment*)	Required (one-layer containment*)	Required (double-layer containment)

The appropriate primary containment for housing arthropods depends on the arthropod's characteristics (flying, crawling, jumping, swimming…) and their requirements (feeding, lighting, temperature, humidity, water…). It should be robust and allow for easy removal and reintroduction of the arthropods without risk of escape.

* The containment stringency should be proportional to the risk (severity and likelihood) for the human health and the environment (e.g. arthropods inoculated with a risk class 3 pathogen). In L2 and L2-Q, where determined by risk assessment, a double layer containment could be required to prevent escape, e.g. in case the number of housed arthropods is too high to allow the control of escapees by counting .

Points of attention:

- *Mesh, when used, should be of an adequate size and robust.*
- *Cages are regularly examined for structural damage and mesh integrity.*
- *The climatic or growing chamber, if used as primary containment to keep arthropods during experiments in the lab, are equipped with a glass door or a mesh door to allow for visual inspection without having to open doors. The climatic or growing chamber may create waste water and infectious aerosols which must be contained in an appropriate way (PPE, HEPA filtered ventilation, waste management, decontamination management…).*
- *It may be necessary to foresee different types of containment for arthropods at different developmental stages.*

40.	**Creation of splashes and aerosols**	Minimized	Minimized	Minimized	Prevented
41.	**Specific measures to control creation of**	Not required	Recommended	Recommended	Required

	splashes and aerosol dissemination				
42.	**Mechanical pipetting**	Required	Required	Required	Required
43.	**Prohibition on drinking, eating, smoking…**	Required	Required	Required	Required
44.	**Appropriate records**	Required	Required	Required	Required
45.	**Check control measures and protective equipment**	Required	Required	Required	Required
46.	**Instructions for use of effective disinfectants** and insecticides/pesticides	Required	Required	Required	Required
Point of attention: *The use of insecticides or pesticides can have harmful long-term effects and should be used as a last resort.*					
47.	**Disinfectants in drains**	Not required	Not required	Not required	Recommended
48 - 49	**Staff training and written biosafety procedures**	Required	Required	Required	Required
	- Separation in time or space of different arthropods	Required	Required	Required	Required
	- Reducing risk of *human* transmission by biting or pricking	Not applicable	Minimize	Not applicable	Prevent
- Separation of arthropods (e.g. to prevent cross-contamination of infected and non-infected arthropods or errors between genetically modified and not genetically modified arthropods) can be done physically but also in time**.** Physical separation between areas is achieved by compartmentation of the space with barriers, corridors, lobbies… Separation in time or space involves careful consideration of the best way of transferring materials and arthropods between containment rooms, a situation that could increase the risk of escape (see SBB document on recommendations for internal transport, in French and Dutch [4]). Separation in time requires also a greater vigilance on waste management when releasing the facility for the other type of arthropod.					

[4] https://www.bioveiligheid.be/sites/default/files/intern_transport_nl.pdf
https://www.biosecurite.be/sites/default/files/transport_interne_fr.pdf

- In the case of human pathogen use and to minimise (L2) or to prevent (L3) the risk of transmission by biting and pricking the following precautions are recommended:
  - apply arthropod anaesthesia or sedation to facilitate safe handling.
  - avoid direct contact by using appropriate containment during housing that allows easy monitoring and/or handling the arthropod without opening the containment (e.g. transparent cage with sleeves).
  - during manipulations, protect bare skin with PPE made of material resistant to bites and pricks.

Point of attention:
When feeding arthropods implies the infestation of living host animals or plants, specific measures to prevent escapees should be implemented based on the risk of the activity. After feeding, animals and plants should be inspected and eventually treated with an insecticide/pesticide for the residual presence of arthropods. Animals and plants, if housed in the same area than arthropods, should be adequately contained to prevent that arthropod escapees can have access to them.

50.	**Efficient vector control (e.g. for rodents and insects)**				
	- Attractants/traps:				
	In the room	Not required	Recommended	Recommended	Required
	In the airlock	Not required	Required if there is an airlock	Required	Required
	In the corridor	Not required	Not required	Required	Required

In the framework of an activity with arthropods, the use of attractants and traps are also directed against arthropods manipulated or maintained in the lab as a preventive measure.

The choice of attractants and traps and their number to be used in routine should be considered according to the characteristics of the arthropods (flying, crawling, jumping…), the room conditions (size, lighting, humidity…) and the risk for human health and the environment. The use of a combination of traps is recommended.
All captured arthropods via these traps are euthanized and disposed of according procedures of infectious waste management.

Examples of traps:
- ovitraps, appendix for depositing mosquito eggs where they can develop but not go out,
- baths of soapy water for flea at ground level,
- oil-filled channels around tick colonies,
- light traps,
- cage in a water bath for nonflying arthropods,
- glue boards,

- (poisoned) food and pheromones traps,
- insectocutors,
- foot bath or sticky mat, fly paper strips,
- …

Points of attention :
- If light (including UV light), pheromones or any other attractant are used as traps they should be located far from the exit of the area to avoid escapees.
- The use of traps gives an overview of how well physical and procedural containment measures are working in the facility. It is therefore necessary to consider:
- the number, the location and the types of traps;
- the frequency of their monitoring and renewal;
- the threshold for taking actions to correct the situation.

51.	**Free-roaming animals**	Forbidden	Forbidden	Forbidden	Forbidden
52.	**In case of zoopathogen manipulation, period of time without any contact with the host animal**	Not required	Not required	Not required	Recommended *

* this period will depend on the infecting zoopathogen and should be determined on a case-by-case basis.

53 - 55	**Waste management:** **Inactivation by an appropriate and validated process of :**				
	- **biological waste and/or residual biological materials before disposal (arthropods and the infecting pathogen);**	Euthanasia and incineration	Euthanasia and incineration	Euthanasia and incineration	Euthanasia of the arthropods and on-site inactivation of the pathogen(s) involved before incineration
	- **contaminated material before wash, reuse or destruction;**	Required	Required	Required	Required
	- **effluents from sinks and showers**	Not required	Not required	Optional	Optional

Management of the arthropod waste should consider the **killing of the arthropods (euthanasia) and the inactivation of the infecting pathogen or GMM**.

Final disposal of this waste is always carried out by incineration according to applicable infectious waste legislation.
Arthropods must undergo a preliminary and adequate chemical or physical treatment (heat, freeze) to kill them before final disposal. This preliminary treatment is necessary to immobilize arthropods and prevent their escape during the inactivation and disposal procedures (e.g. escape from the autoclave, the bin). In the case of biologically contaminated liquids (e.g. effluents from aquariums, irrigation water from plants, water from equipment reservoirs or from devices such as air dehumidification...), it may be opted to filter first to facilitate the inactivation of the harmful arthropods,
In L3, all biological waste including pathogens, GMMs and infected arthropods are inactivated on-site before final disposal by incineration.
All inactivating methods and killing treatments should be validated specifically for each type of organism.

Points of attention:

- *Chemicals or gaseous disinfectants used to inactivate pathogens may be ineffective to kill arthropods. Validated thermal inactivation (e.g. autoclave) guarantees the inactivation of pathogens and the destruction of arthropods.*
- *If the autoclave is outside the containment, safe transport procedure for waste is foreseen.*
- *Euthanasia by crushing is allowed for GM arthropods and Q arthropods provided they are not contaminated with pathogenic or Q organisms.*

Emergency procedures Adequate insecticides or pesticides available	Required	Required	Required	Required

Procedures and emergency measures should be established to manage incidents and emergencies due to the failure of primary or secondary containment (such as a break in the facility shell, primary containment, HVAC, screens…) that could lead to a significant release of arthropods.
A hoover, a fly swatter, a CO_2 gas bottle or, as a last resort, an insecticide should be foreseen in the facility to prevent the possible escape of arthropods into the environment.
Keep in mind that these actions may not inactivate the pathogenic micro-organism, hence recaptured arthropods should be collected and disposed of as infectious waste according to applicable waste law(s).

Points of attention:

- *In an emergency situation (large and uncontrolled release of arthropods), application of an insecticide or pesticide may be necessary. Careful consideration should be given to the impact this use may have such as the potential insecticide toxicity for other arthropods housed in the facility or suspension of activities in the treated rooms,…*
- *It is recommended to frequently change the type of disinfectants and insecticide/pesticide to avoid resistance development.*

Additional work practices specific to arthropods				
- Monitoring and controlling numbers of arthropods	Not required	Not required	Required	Required
- “Buddy principle” (or work in pairs)	Not required	Not required	Optional	Optional

- A way of arthropods control is tracking the numbers of arthropods housed within a primary containment. This is facilitated if the number of arthropods used is limited. Accurate counting and recording at each stage of handling is preferred until final disposal of the arthropods to detect any losses. If the numbers are no longer correct and the missing arthropods cannot be found and captured immediately emergency procedures must be activated to mitigate the possible biological risk caused by the escape.
In case the control of escapees is not possible by counting (because the number of arthropods is too high for instance), multiple physical barriers between arthropods and lab space can be used to render the way of exit too difficult for arthropods.

- A **"**buddy system" or working in pairs consists of a collaboration between two trained workers to carry out delicate operations. It can reduce the risk of certain tasks and errors with arthropods by providing direct practical support for the study or seeking assistance in the event of an emergency.

Animal houses (A) or dedicated facilities for housing and rearing arthropods

Notes:

The following criteria apply to the containment required to house and/or rear arthropods in the framework of a contained use.
The levels of containment are defined as follows:
A1 for containment level (CL)1 activities involving genetically modified (GM) arthropods or arthropods (GM or not) inoculated with non-pathogenic GM micro-organisms.
A2 for CL2 activities involving arthropods (GM or not) inoculated with a pathogen (GM or not) of minimum class of risk 2.
A3 for CL3 activities involving arthropods (GM or not) inoculated with a pathogen (GM or not) of minimum class of risk 3.

There is no A2-Q containment level defined in regional legislation on contained use (CU) of GM organisms and/or pathogens. If quarantine (Q) arthropods or arthropods inoculated with Q organisms are reared and kept in a specific room, the level of containment of this room will be determined on a case-by-case basis, with minimal A2 requirements.

Design and technical specifications

Specifications		A1	A2	A3
1.	**Animal house suite: isolation**	Not required	Required	Required
2.	**Access via airlock**	Not required	Recommended	Required
Comment: *When working with arthropods, the airlock has the following advantages :* ▪ *it acts as a physical barrier to potentially escaped arthropods. Additionally, traps can be installed in it to reinforce prevention of escapees into the environment.* ▪ *it facilitates the possibility for workers to check for any escapees hanging on their clothes and on other personal protective equipment (PPE) before final exit. The use of mirrors allows visual inspection of large enough arthropods on clothes or PPE.* ▪ *where pertinent (cold sensitive arthropods), it can be permanently set at a controlled temperature at which arthropods cannot survive or it can be cooled periodically to such temperatures, especially in the event of a known/suspected escape. Alternatively or in addition to the cooled airlock, corridors may also be cooled.* ▪ *An alternative to a permanently built airlock consists in placing a meshed space at the entrance, at least if enough space is available.*				
3.	**Lockable access door(s)**	Required	Required	Required

4.	**Self-closing access door(s)**	Not required	Required	Required
This system contributes also in preventing escapes. Door should close quickly.				
5.	**Sealed windows**	Not required	Recommended to close during experiments*	Required
* If the A2 is used to house Q arthropods or arthropods infected with Q organisms, windows are permanently sealed. A screen or mosquito net should be placed on windows that can be opened (A1, A2) . Where nets are used, they should be of an appropriate sized mesh to stop the involved arthropods and robust enough to withstand airflows.				
6.	**Room sealable for fumigation**	Not required	Optional	Required
7.	**Building designed to prevent accidental escape of animals**	Recommended	Required	Required
8.	**An observation window or alternative so that occupants can be seen**	Recommended	Recommended	Required
A window in the entrance door or equivalent (e.g. a camera) allows to see who is in the room as well as to assess the general situation in the animal house. This is compulsory if there is a risk of incident by opening the door. *Point of attention:* *Light cycle is sometimes necessary to apply for adequate maintenance of arthropods in the animal house. A device (a mobile shield for instance) on the windows to hide the outside light can be foreseen. This measure is also useful to prevent light attraction of flying arthropods.*				
9.	**Access for staff to washing and decontamination facilities**	Required	Required	Required
If required by risk assessment and as a last resort (accidental release), suitable insecticides or pesticides are poured in siphons and water traps. In A2 and A3, seal drains (sink, floor, shower) with an adapted mesh to prevent escape of arthropods. The drain of the sink should be covered with a filter or fine mesh to avoid the escape of any eggs or larvae.				

10.	**Non-hand operated sink**	Not required	Recommended	Required
11.	**Coat hangers or lockers for protective clothing** In airlock when available	Required	Required	Required
12.	**Fluid supply lines are fitted with backflow prevention devices**	Not required	Not required	Recommended
13.	**Separate room for storage of clean cages, feed and bedding**	Recommended	Required	Required
14.	**Surfaces resistant to disinfectants and decontamination agents, waterproof and easy to clean** In addition : - resistant to insecticides/pesticides - light coloured - smooth and seamless	Required for cages, benches Required Required Not required	Required for cages, benches and floor Required Required Optional	Required for cages, benches, floor, walls and ceiling Required Required Required
	Surfaces including those of benches, furniture, walls, floor, ceiling, are contrasting in colour to facilitate detection of escaped arthropods. In A3, and A2 when Q arthropods or arthropods inoculated with Q organisms are housed, surfaces should be smooth and seamless with no cracks or crevices, as these provide hiding places for escaped (or entering) arthropods.			
15.	**Cages washing facility**	Required	Required	Required
16.	**Autonomous electric system**	Not required	Not required	Recommended
17.	**Fire detection and alarm system**	Not required	Not required	Required
18.	**System to communicate with outside the contained zone**	Not required	Not required	Required

	<u>Ventilation</u>			
19.	**Supply air ducts independent from those in adjacent rooms**	Not required	Not required	Recommended
20.	**Exhaust air ducts independent from those in adjacent rooms**	Not required	Optional	Recommended
21.	**Supply and exhaust air ducts interconnected**	Not required	Optional	Required
22.	**Supply and exhaust air systems that can be closed by means of valves**	Not required	Optional	Required
23.	**Negative pressure relative to the pressure of the immediate environment**	Not required	Optional	Required
24.	**Air from the room should be HEPA filtered**	Not required	Optional	Required (exhaust air)
25.	**System to change filters safely**	Not applicable	Required if filters present	Required
26.	**Filtered air reusable**	Not applicable	Optional	Optional
27.	**Specific measures to adequately ventilate the area**	Optional	Optional	Required
<u>Additional measures specific to arthropods</u> **aiming to control possible "hot spots" for arthropod escape :** - Screened and/or sealed : grids, holes, gaps, key holes… air inlets/outlets pipework (drainage, sink…)		 Not required Not required Not required	 Optional Optional Optional	 Required Required Required

electrical service conduits (socket outlets, lighting…)	Not required	Optional	Required
- Low ceiling height	Not required	Optional *	Required

- To prevent escapees in A3 and A2 when determined by risk assessment (e.g. Q arthropods or arthropods infected with Q organisms or with pathogens of risk class 3), screen or seal any hot spots for arthropod escape (e.g. grids, holes, gaps…) in the facility. Where screens are used, they should be of an appropriate sized mesh to stop the involved arthropods and robust enough to withstand airflows.
The entrance door is tight-fitting and when closed, all free-spaces or crevices are sealed or covered using magnetic seal strips, brush barriers, flexible flanges or equivalent. Key holes on doors are avoided or adequately sealed.
Air inlets/extracts, all pipe work (e.g. drainage, sink) and electrical service conduits are sealed to prevent escapees. Ceiling mounted lighting should be flushed with the ceiling and ideally accessible from above to avoid breaching containment.

- Having low ceiling heights makes it easier to detect escaped arthropods as well as their recapture. Captured arthropods should be disposed of according procedures of infectious waste management.
* For instance, it is recommended to house Q arthropods and arthropods infected with Q organisms in a A2 with a low ceiling.

Points of attention:

- *Achieve regularly an in-depth inspection of the physical containment (e.g. walls, screens, seals, mounted lights). It is also recommended to check "hot spots for escape" weekly or even daily. The frequency at which hot spots must be checked is evaluated on a case-by-case basis. Replacement must be foreseen where necessary;*
- *These measures will also prevent entry of arthropods and other unwanted animals into the room.*

Safety Equipment

Specifications		A1	A2	A3
28.	**Biosafety cabinet**	Not required	Optional	Optional

The use of a biosafety cabinet (BSC) may be difficult with small arthropods that cannot withstand the strong airflow within the cabinet. In this case a BSC is only used to prepare the infectious material that will be inoculated to the arthropods. Then, if a primary containment is necessary during infection and handling of infected arthropods, a glovebox (without laminar airflow) can be used to isolate and allow safe manipulations without risk of escape. Immobilization (physical, cold or via anaesthesia) can be applied as an alternative. If during handling exposure cannot be excluded and if adequate immobilization cannot be foreseen, a biosafety cabinet or an (HEPA filtered) isolator is required. Appropriate sized glovebox can be used inside the BSC to protect arthropods from BSC airflow.

An insecticide, pesticide or a CO_2 gas bottle is foreseen inside the device in the case of an emergency.

29.	**Arthropods kept in cages or equivalent appropriate containment facilities (pens or tanks,…)**	Required	Required	Required
30.	**Isolators with HEPA filter**	Not required	Optional	Required
31.	**Autoclave**	On site	In the building	In room or annex *
* with validated procedures allowing the safe transfer of the biological material to an autoclave outside the room and offering an equivalent level of protection.				
32.	**Double-ended autoclave**	Not required	Not required	Recommended
33.	**Fumigation device, freezing or disinfectant bath**	Not required	Recommended	Required
Additional specific equipment specific to arthropods				
Air curtain at the entrance door of the room		Not required	Optional	Optional
An air curtain (or equivalent) creates an impenetrable screen of air for flying or light arthropods [12].				

Work practices and waste management

Specifications		A1	A2	A3
34.	**Restricted access** Controlled access	Required Not required	Required Optional	Required Required
35.	**Indications on the door:** a. Biohazard sign; b. contact details of the person in charge; c. containment level; d. nature of the biological risk; e. authorized access persons; f. access criteria (e.g. specific training)	b, c, d, e, f	a, b, c, d, e, f	a, b, c, d, e, f

36.	**Room contains its own equipment** **Reduce work flow** (material, personnel and arthropods)	Not required Not required	Recommended Minimized	Required Minimized
37 - 41	**Personal protection equipment (PPE) :** Coat specific to the contained area Coat decontaminated before exit from the area Gloves Respiratory protection Face protection	 Required Not required Optional Not required Not required	 Required Optional * Recommended Optional Optional	 Required Required Required Optional Optional

* Wearing a protection coat is mandatory in all containment levels. In A3 and, where determined by risk assessment in A2 (e.g. housing Q arthropods or arthropods infected with Q organisms), coat should be decontaminated before leaving the area.
Other personal protection equipment (PPE) should be selected taking into account the risk level of the activity, the mode of transmission of the pathogens or GMMs infecting the arthropods and the physical barriers already in place in the facility.
PPE can prevent unintentional release of an arthropod by protecting the hair, covering shoes… or by preventing access to any hiding place. For this reason, in A3, PPE should be specific to the animal house. This measure should also be followed in A2 when determined by risk assessment (e.g. housing Q arthropods or arthropods infected with Q organisms).

Procedures for staff should be in place to describe the removal and treatment of PPE.

Points of attention:

- *PPE are preferably contrasting of colour for easy detection of arthropods*.
- *As PPE is a possible escape route for arthropods that may linger or hide in it, it is systematically checked before removal, with or without the use of mirrors, preferably in the airlock if available.*
- *A hand-size vacuum foreseen in the airlock can be used to remove possible arthropods hanging on PPE and on areas that were not protected by PPE. Recovered organisms are disposed of according procedures of infectious waste management.*
- *It is useful to wear appropriate PPE that prevents escape such as lab coats without pockets, with zips, cuffed sleeves and trouser legs, etc.*
- *To further reduce this risk, it may be considered to freeze the PPE (-15°C or lower depending on the arthropod sensibility to cold) while awaiting reuse.*

42.	**Creation of splashes and aerosols**	Minimized	Minimized	Prevented
43.	**Specific measures to control creation of splashes and aerosol dissemination**	Not required	Recommended	Required

44.	**Mechanical pipetting**	Required	Required	Required
45.	**Ban on drinking, eating, smoking, …**	Required	Required	Required
46.	**Appropriate records of operations with arthropods**	Required	Required	Required
47.	**Check control measures and protective equipment**	Required	Required	Required
48.	**Instructions for use of effective disinfectants** and insecticides/pesticides	Required	Required	Required

Point of attention:
The use of insecticides or pesticides can have harmful long-term effects and should be used as a last resort.

49.	**Disinfectants in drains**	Not required	Recommended	Recommended
50 - 51	**Staff training and written biosafety procedures**	Required	Required	Required
	- Separation in time or space of different arthropods	Required	Required	Required
	- Reducing risk of *human* transmission by biting or pricking	Not applicable	Minimize	Prevent

- Separation of arthropods e.g. to prevent cross-contamination of infected and non-infected arthropods, genetically modified and not genetically modified arthropods, can be done physically but also in time**.**
Physical separation between these areas is achieved by compartmentation with barriers, corridors, lobbies.
Separation in time or space involves careful consideration of the best way of transferring materials and arthropods between containment rooms, a situation that could increase the risk of escape (see SBB document on recommendations for internal transport, in French and Dutch [5]). Separation in time requires also a greater vigilance on waste management when releasing the facility for another arthropod.

- When using human pathogens and to minimise (A2) or prevent (A3) the risk of transmission by biting and pricking, the following precautions are recommended:
- apply arthropod anaesthesia or sedation if needed.
- avoid direct contact by using appropriate containment during housing that allows easy monitoring and/or handling the arthropod without opening the containment (e.g. transparent insectaria with sleeves).

[5] https://www.bioveiligheid.be/sites/default/files/intern_transport_nl.pdf
https://www.biosecurite.be/sites/default/files/transport_interne_fr.pdf

- during manipulations, protect bare skin with PPE made of material resistant to bites and pricks.

Points of attention:
When feeding arthropods implies the infestation of living host animals or plants, specific measures to prevent escapees should be placed based on the risk of the activity. After feeding, animals and plants should be inspected and eventually treated with an insecticide/pesticide for the residual presence of arthropods. Animals and plants, if housed in the same area than arthropods, should be adequately contained to prevent their access to potential arthropod escapees.

52.	**Efficient vector control (e.g. for rodents and insects)**			
	- Attractants/traps:			
	In the room	Recommended	Required	Required
	In the airlock	NA	Required	Required
	In the corridor	Not required	Not required	Required

In the framework of an activity with arthropods, the use of attractants and traps should also be directed against arthropods reared in the animal house as a preventive measure.

The choice of attractants and traps and their number to be used in routine should be considered according to the characteristics of the arthropods (flying, crawling, jumping…), the room conditions (size, lighting, humidity,…) and the risk for human health and the environment. The use of a combination of traps is recommended. All captured arthropods via these traps are disposed of according procedures of infectious waste management.

Examples of traps:
- ovitraps, appendix for depositing mosquito eggs where they can develop but not go out,
- baths of soapy water for flea at ground level,
- oil-filled channels around tick colonies,
- light traps for mosquitoes,
- cage for nonflying arthropods deposited in a water bath,
- glue boards,
- light,
- (poisoned) food and pheromones traps,
- insectocutors,
- foot bath or sticky mat, fly paper strips,
- …

Points of attention :
- If light (including UV light), pheromones or any other attractant are used as traps they should be located far from the exit of the area to avoid escapees.
- The use of traps gives an overview of how well physical and procedural containment measures are working in the facility. It is therefore necessary to consider:
- the number, the location and the types of traps;

- the frequency of their monitoring and renewal;
- the threshold for taking actions to correct the situation.

53.	**Isolation of experimental arthropods**	Required	Required (separated room)	Required (separated room)
54.	**In case of zoopathogen manipulation, period of time without any contact with host animal** This period will depend on the infecting zoopathogen and should be determined on a case-by-case basis.	Not required	Not required	Recommended
55 - 57	**Waste management: inactivation by an appropriate and validated process of**			
	- biological waste and/or residual biological materials before disposal	Euthanasia and incineration	Euthanasia and incineration	Euthanasia of the arthropods (and on-site inactivation of the pathogen(s)) involved before incineration
	- contaminated material before wash, reuse or destruction	Optional	Required	Required
	- effluents from sinks and showers	Not required	Not required	Recommended

Management of the arthropod waste should consider the **killing of the arthropods and the need of inactivation of the infecting pathogen or GMM.**
Final disposal of this waste is always carried out by incineration according to applicable infectious waste legislation.
Arthropods must undergo a preliminary and adequate chemical or physical treatment (heat, freeze) to kill them before final disposal. This preliminary treatment is necessary to immobilize arthropods and prevent their escape during the inactivation and disposal procedures (e.g. escape from the autoclave, the bin)

In the case of biologically contaminated liquids (e.g. effluents from aquariums, irrigation water from plants, water from equipment reservoirs, devices such as air dehumidification...), it may be opted to filter first to facilitate the inactivation of the harmful arthropods,
In A3, all biological waste including pathogens, GMMs and infected arthropods are inactivated on-site before final disposal by incineration.

All inactivating methods and killing treatments should be validated specifically for each type of organism.

Points of attention:

- *Chemical or gaseous disinfectants used to inactivate pathogenic micro-organisms may be ineffective to kill some arthropods. Thermal inactivation (autoclave) under the appropriate conditions guarantees the inactivation of pathogens and the destruction of arthropods.*
- *If the autoclave is outside the containment, safe transport procedure for waste should be foreseen.*
- *Euthanasia by crushing is allowed for GM arthropods and Q arthropods provided they are not contaminated with pathogenic or Q organisms.*

Emergency procedures Adequate insecticides/pesticides available	Required	Required	Required

Procedures and emergency measures should be established to manage incidents and emergencies due to the failure of primary or secondary containment (such as a break in the facility shell, primary containment, HVAC, screens…) that could lead to a significant release of arthropods. These measures (equipment to recapture, such as a hoover, fly swatter, CO2 gas bottle or, as a last resort, insecticide,...) …) should prevent the possible escape of arthropods into the environment. Preventively, the area is equipped with adequate traps. Keep in mind that these actions may not inactivate the pathogenic micro-organism in question, hence recaptured arthropods should be collected and disposed of as infectious waste according to applicable waste law(s).

Points of attention:

- *In an emergency situation (large and uncontrolled release of arthropods), application of an insecticide or pesticide may be necessary. Careful consideration should be given to the impact of the insecticides or pesticides used on the arthropods housed elsewhere in the facility due to the necessary downtime of the room before experimenting again with arthropods.*
- *It is recommended to frequently change the type of disinfectants and insecticide/pesticide to avoid resistance development.*

Additional work practices specific to arthropods			
- Physical containment (closed cage system)	Recommended (one-layer containment)	Required (one-layer containment)*	Required (double-layer containment)
- Monitoring and controlling numbers of arthropods	Not required	Optional	Required
- « Buddy principle » (or work in pairs)	Not required	Optional	Optional

- The primary containment for housing arthropods should be appropriate depending on the arthropod's characteristics (flying, crawling, jumping, swimming…) and their requirements (feeding, lighting, temperature, humidity…). It should allow for easy removal and reintroduction of the arthropods without risk of escape.

* The containment stringency should be proportional to the risk for the human health and the environment. In A2 where determined by risk assessment, escape

prevention must be additionally ensured by a double layer containment.

Points of attention:

- *Mesh, when used, should be of an adequate size and robust.*
- *Cages should be visually examined regularly for structural damage and mesh integrity.*
- *The climatic and/or growing chamber, if used as primary containment, should be equipped with a glass door or a mesh door to allow for visual inspection without having to open doors. The climatic and/or growing chamber may create waste water and infectious aerosols which must be contained in an appropriate way (PPE, HEPA filtered ventilation, waste management, decontamination management…).*
- *It may be necessary to foresee different types of primary containment for arthropods at different developmental stages.*

- A way of arthropods control is tracking the numbers of arthropods housed within a primary containment. This is facilitated if the number of arthropods housed is limited. Accurate counting and recording at each stage of handling is preferred until final disposal of the arthropods to detect any losses. If the numbers are no longer correct and the missing arthropods cannot be found and capture immediately, emergency procedures must be activated to mitigate the possible biological risk caused by the escape.

In case the control of escapees is not possible by counting (because the number of arthropods is too high for instance), multiple physical barriers between arthropods and the animal house environment can be used to make difficult the way of exit.

- A **"**buddy system" or working in pairs consists of a collaboration between two trained workers to carry out delicate operations. It can reduce the risk of certain tasks and errors with arthropods by providing direct practical support for the study or seeking assistance in the event of an emergency.

Greenhouses and growth rooms (G) or dedicated contained area for rearing and housing of arthropods on plants

Notes:

The following criteria apply to the containments of arthropods reared or maintained on plants.
Greenhouses and growing rooms are defined as structures with walls, a roof and a floor, intended primarily for growing plants in a controlled and protected environment.

The levels of containment are defined as follows:
G1 for containment level (CL) 1 activities of risk class 1 involving the rearing or housing on plants of genetically modified (GM) arthropods or arthropods (GM or not) inoculated with non-pathogenic GM micro-organisms.
G2 for CL2 activities of risk class 2 involving the rearing or housing on plants of arthropods (GM or not) inoculated with a pathogen (GM or not) of minimum class of risk 2.
G2-Q for CL2-Q activities involving the rearing or housing on plants of quarantine (Q) arthropods or arthropods (GM or not) inoculated with a Q organism.
G3 for CL3 activities of risk class 3 involving the rearing or housing on plants of arthropods (GM or not) inoculated with a pathogen (GM or not) of minimum class of risk 3.

Design and technical specifications

Specifications		G1	G2	G2-Q	G3
1.	**Greenhouse is a permanent structure**	Not required	Required	Required	Required
Except in G1, the greenhouse must be a permanent structure with a continuous waterproof roof, located on a sloping site so as to prevent surface run-off penetration and with self-closing lockable doors. Taking into account biological risks of the housed arthropods, greenhouse location is in an area that presents minimal risks to the environment (agriculture, livestock, forests). An alternative to an isolated area is the increase of physical barriers containing the arthropods.					
2.	**Area around the greenhouse is free for 1.5 meters (of debris and vegetation)**	Not required	Required	Not required*	Required
* the Belgian CU legislation does not require this measure in context of a contained use of quarantine organisms given that windows cannot be opened and because of the presence of an airlock. This kind of area can harbour traps or sentinel plants which help to monitor potential escapees.					

3.	**Security fence**	Not required	Not required	Not required	Required
4.	**Aisles**	Stabilized	Solids	Solids	Solids
5.	**Entrance via an airlock** **Two interlocking doors are foreseen to prevent them from being opened at the same time**	Not required	Optional	Required	Required

Doors are self-closing and close rapidly.

Comment:
When working with arthropods, the airlock has the following advantages :

- *it acts as a physical barrier to potentially escaped arthropod. Additionally, placing traps in it reinforces prevention of escapees into the environment;*
- *it facilitates the possibility for workers to check for any escapees hanging on clothes and on other personal protective equipment (PPE) before final exit. The use of mirrors allows visual inspection of (large enough) arthropods on PPE ;*
- *where pertinent (cold sensitive arthropods) it can be permanently set at a controlled temperature at which the arthropod cannot survive or it can be cooled periodically to such temperatures, especially in the event of a known/suspected escape. Alternatively or in addition to cooled airlock and airlock, corridors may also be cooled.*
- *An alternative to a permanently built airlock consists in placing a meshed space at the entrance, at least if enough space is available.*

6.	**Lockable access door**	Not required	Required	Required	Required
7.	**Structure resistant to impact / collision (wall, floor, roof)** as well as to frequent adverse weather conditions (in area with high winds, flooding,…)	Not required	Recommended	Recommended	Recommended
8.	**Structure resistant to water and easy to clean**	Not required	Recommended	Required	Required
9.	**Sealed windows**	Not required	Not required	Required	Required

In G1 and G2, a screen or mosquito net should be placed on windows that can be opened. Where nets are used, they should be of an appropriate sized mesh to stop the involved arthropods and robust enough to withstand airflows.

10.	**Greenhouse sealable for fumigation**	Not required	Not required	Required	Required
11.	**Access for staff to washing and decontamination facilities**	Required (sink)	Required (sink)	Required (sink)	Required (sink in airlock or next to exit)

In G2-Q and G3, seal sewer or drains (sink, floor, shower, etc.) with an adapted mesh to prevent escape of arthropods.
If required by risk assessment and as a last resort (in case of large accidental release), suitable insecticide or pesticide is poured in siphons and water traps.

12.	**Non-hand operated sink**	Not required	Not required	Optional	Required
13.	**Fluid supply lines are fitted with backflow prevention devices**	Not required	Not required	Not required	Recommended
14.	**Surfaces resistant to acids, alkalis, organic solvents, disinfectants**	Not required	Recommended	Recommended	Required
	In addition :				
	- resistant to insecticides or pesticides	Required	Required	Required	Required
	- light coloured	Required	Required	Required	Required
	- smooth and seamless	Not required	Not required	Required	Required

Surfaces including those of benches, furniture, walls, floor, ceiling are contrasting in colour to facilitate detection of escaped arthropods. In G3 and G2-Q, surfaces are smooth and seamless with no cracks or crevices, as these provide hiding places for escaped (or entering) arthropods.

15.	**Waterproof floor**	Not required	Recommended	Required	Required
16.	**Control of contaminated run-off water**	Optional	Minimized flow*	Prevent flow*	Prevent flow

* When transmission can occur through the ground.

17.	**Autonomous electric system**	Not required	Not required	Not required	Required
18.	**Fire detection and alarm system**	Not required	Optional	Optional	Required

19.	**System to communicate with outside zone**	Not required	Optional	Optional	Required
	<u>Ventilation</u>				
20.	**Supply and exhaust air ducts interconnected to prevent accidental overpressure**	Not required	Optional	Required	Required
21.	**Supply and exhaust air systems that can be closed by means of valves**	Not required	Optional	Optional	Required
22.	**Negative pressure relative to the pressure of the immediate environment**	Not required	Not required	Optional	Optional
23.	**Extract and input air should be HEPA filtered**	Not required	Not required	Optional (extraction)	Required
24.	**System to change filters safely**	NA	NA	Optional	Required
<u>Additional measures specific to arthropods</u> aiming to control possible "hot spots" for arthropod escape:					
Screened and/or sealed :					
	grids, holes, gaps, key holes…	Not required	Optional	Required	Required
	air inlets/outlets	Not required	Optional	Required	Required
	pipework (drainage, sink…)	Not required	Optional	Required	Required
	electrical service conduits (socket outlets, lighting…)	Not required	Optional	Required	Required
Low ceiling height		Not required	Optional	Required	Required

To prevent escapees in G2-Q and G3 and in G2 when determined by risk assessment (e.g. arthropods infected with a pathogen of risk class 3), screen or seal any hot spots for arthropod escape (e.g. grids, holes, gaps…) in the facility. Where screens are used, they should be of an appropriate sized mesh to stop the involved arthropods and robust enough to withstand airflows.

The entrance door is tight-fitting and when closed, all free-spaces or crevices are sealed or covered using for instance magnetic seal strips, brush barriers, flexible flanges or equivalent. Key holes on doors are avoided or adequately sealed.
Air inlets/extracts, all pipe work (e.g. drainage, including in growth chamber, sink) and electrical service conduits are sealed to prevent escapees. Ceiling mounted lighting should be flushed with the ceiling and ideally accessible from above to avoid breaching containment.
Having low ceiling heights makes easier the detection of escaped arthropods as well as their recapture. Captured arthropods should be disposed of according procedures of infectious waste management.

Points of attention:
Achieve regularly an in-depth inspection of the physical containment (e.g. walls, screens, seals, mounted lights). It is also recommended to check "hot spots for escape" weekly or even daily. The frequency at which hot spots must be checked is evaluated on a case-by-case basis. Replacement must be foreseen where necessary.
These measures will also prevent entry of arthropods and other animals into the room.

Safety Equipment

Specifications		G1	G2	G2-Q	G3
25.	**Autoclave**	On site	In the building	In greenhouse or annex *	In greenhouse
* with validated procedures allowing the safe transfer of the material to an autoclave outside the greenhouse and offering an equivalent level of protection.					
26.	**Double-ended autoclave**	Not required	Not required	Not required	Optional
27.	**Fumigation chamber or soak tank for the transfer of live material**	Not required	Not required	Optional	Optional
Additional safety equipment specific to arthropods					
Air curtain at entrance door of the greenhouse or growth room		Not required	Optional	Optional	Optional
An air curtain (or equivalent) creates an impenetrable screen of air for flying or light arthropods [12].					

Work practices and waste management

Specifications		G1	G2	G2-Q	G3
28.	**Restricted access** **Controlled access**	Required Not required	Required Optional	Required Optional	Required Required
In G3, a controlled access system such as the use of an electronic access card for authorized and trained staff is mandatory. In G2 and G2-Q, it may be mandatory when specific training related to housed arthropods is required.					
29.	**Indications on the door:** a: Biohazard sign b: contact details of the person in charge c: containment level d: nature of the biological risk e: authorized access persons f: access criteria (e.g. specific training)	Not required	a, b, c, d	a, b, c, d	a, b, c, d, e, f
30.	**Greenhouse to contain its own equipment** **Reduce work flow** (material, personnel and arthropods)	Not required Not required	Not required Minimized	Required Minimized	Required Minimized
31 - 34	**Personal protective equipment (PPE) :** Coat Coat decontaminated before leaving the greenhouse Gloves Shoe cover or decontamination bath for shoes or sticky mats	 Required Not required Not required Not required	 Required Not required Optional Optional	 Required and specific to the contained area * Recommended Optional Optional	 Required and specific to the contained area Required Optional Optional

* Although not required in G2-Q by the CU legislation, it is strongly recommended to wear a coat specific to the contained area as it is for G3, conform the online document "Working practices on the use of protective clothing"[6].

PPE can prevent unintentional release of an arthropod by protecting the hair, covering shoes,… or by preventing access to any hiding place.

Procedures for staff should be in place to describe the removal and treatment of PPE.

Points of attention:

- *Coats are preferably white or light of colour*.
- *As PPE is a possible escape route for arthropods that may linger or hide in it, their systematic check when leaving the greenhouse before removal is performed in all containment levels except G1, with or without the use of mirrors, preferably in the airlock.*
- *A hand-size vacuum can be foreseen (in the airlock if available) to remove possible arthropods on PPE and on area's that were not protected by PPE. Recovered organisms are disposed of following procedures of infectious waste management.*
- *It is useful to wear appropriate PPE that prevents escape such as lab coats without pockets, with zips, cuffed sleeves and trouser legs, etc.*
- *To further reduce this risk, it may be considered to freeze the PPE (-15°C or lower depending on the arthropod resistance to cold) while awaiting reuse.*

35.	**Splashes and aerosol creation**	Minimized	Minimized	Prevented	Prevented
36.	**Specific measures to control splash creation and aerosol dissemination**	Not required	Recommended	Recommended	Required
37.	**Mechanical pipetting**	Required	Required	Required	Required
38.	**Ban on drinking, eating, smoking…**	Required	Required	Required	Required
39.	**Appropriate record of operations**	Required	Required	Required	Required
40.	**Check control measures and protective equipment**	Required	Required	Required	Required
41.	**Instructions for use of effective disinfectants** and insecticides/pesticides	Required	Required	Required	Required

Point of attention:

[6] NL : https://www.bioveiligheid.be/sites/default/files/werkpraktijken_beschermkledij.pdf
FR : https://www.biosafety.be/sites/default/files/pratique_trav_vetement_protection.pdf

The use of insecticides or pesticides can have harmful long-term effects and should be used as a last resort.

42	**Staff training and written biosafety procedures**	Required	Required	Required	Required
43	Separation in time or space of different arthropods	Required	Required	Required	Required

Separation of arthropods e.g; to prevent cross-contamination of infected and non-infected arthropods, genetically modified and not genetically modified arthropods, can be done physically but also in time**.**
Physical separation between these areas is achieved by compartmentation with barriers, corridors, lobbies.
Separation in time or space involves careful consideration of the best way of transferring materials and arthropods between containment rooms, a situation that could increase the risk of escape (see SBB document on recommendations for internal transport, in French and Dutch [7]). Separation in time requires also a greater vigilance on waste management when releasing the facility for another arthropod.

44.	**Free-roaming animals**	Forbidden	Forbidden	Forbidden	Forbidden
45.	**Measures to control undesired species such as insects, rodents, arthropods**	Required	Required	Required	Required
	- Attractants/traps:				
	In the greenhouse	Not required	Recommended	Recommended	Required
	In the anteroom	NA	Required if there is an anteroom	Required	Required

In the framework of an activity with arthropods, the use of attractants and traps are also directed against arthropods manipulated or reared in the greenhouse.
The choice of attractants and traps and their number to be used in routine should be considered according to the characteristics of the arthropods (flying, crawling, jumping…), the room conditions (size, lighting, humidity…) and the risk for human health and the environment. The use of a combination of traps is recommended.
All captured arthropods via these traps are euthanized and disposed of according procedures of infectious waste management.

Examples of traps:
- ovitraps, appendix for depositing mosquito eggs where they can develop but not go out,

[7] https://www.bioveiligheid.be/sites/default/files/intern_transport_nl.pdf
https://www.biosecurite.be/sites/default/files/transport_interne_fr.pdf

- baths of soapy water for flea at ground level,
- oil-filled channels around tick colonies,
- light traps for mosquitoes,
- cage for nonflying arthropods deposited in a water bath,
- glue boards,
- light,
- (poisoned) food and pheromones traps,
- insectocutors,
- foot bath or sticky mat, fly paper strips,
- …

Points of attention :
- If light (including UV light), pheromones or any other attractant are used as traps they should be located far from the exit of the area to avoid escapees.
- The use of traps gives an overview of how well physical and procedural containment measures are working in the facility. It is therefore necessary to consider:
- the number, the location and the types of traps;
- the frequency of their monitoring and renewal;
- the threshold for taking actions to correct the situation.

46.	**Disseminating organisms:** - **Transport inside facility between contained zones in a container** - **Transport recorded** - **Decontamination of transport containers**	Optional Not required Not required	Required Recommended Required	Required Required Required	Double container Required Required
47.	**Control of contaminated run-off water** For instance, by sloped floors toward drains and curbs to contain water and collect and treat water	Optional	Water run-off minimized	Water run-off prevented	Water run-off prevented
48 – 50	**Waste management: inactivation by an appropriate and validated process of :** - **biological waste and/or residual biological materials (arthropods, pathogens, plants, contaminated substrate…) before disposal;**	Required	Required	Required	Required

	- **contaminated material before wash, reuse or destruction;**	Required	Required	Required	Required
	- **effluents from sinks and showers**	Not required	Not required	Optional	Optional

Management of the arthropod waste should consider the **killing of the arthropods (euthanasia) and the inactivation of the infecting pathogen or GM micro-organism**. Final disposal of arthropod waste is always carried out by incineration according to applicable infectious waste legislation.
Arthropods must undergo a preliminary and adequate chemical or physical treatment (heat, freeze) to kill them before final disposal. This preliminary treatment is necessary to immobilize arthropods and prevent their escape during the inactivation and disposal procedures (e.g. escape from the autoclave, the bin). In the case of biologically contaminated liquids (e.g. effluents from aquariums, plants, water from equipment reservoirs, devices such as air dehumidification...), it may be opted to filter first to facilitate the inactivation of the harmful arthropods.
In G3, all biological waste including plants, pathogens, GM micro-organisms and infected arthropods are inactivated on-site before final disposal by incineration.
All inactivating methods and killing treatments should be validated specifically for each type of organism.

Points of attention:

- *Chemical or gaseous disinfectants used to inactivate pathogens may be ineffective in killing arthropods and requires other inactivation methods, such as thermal inactivation (e.g. autoclave, steaming).*
- *If the autoclave or steam installation is outside the containment, safe contained transport procedure for waste is foreseen.*
- *Euthanasia by crushing is allowed for GM arthropods and Q arthropods provided they are not contaminated with pathogenic or Q organisms.*
- *If plants can be cleared of arthropods and are not infected by a pathogen, the plant material can be disposed of by means other than incineration, for example for composting. This does not apply to genetically modified plants that are likely to spread or to plants from a G3.*

Emergency procedures Adequate insecticides or pesticides available	Required	Required	Required	Required

Procedures and emergency measures should be established to manage incidents and emergencies due to the failure of primary or secondary containment (such as a break in the facility shell, primary containment, HVAC, screens…) that could lead to a significant release of arthropods. These measures (equipment to recapture, such as a hoover, fly swatter, CO2 gas bottle or, as a last resort, insecticide,...) should prevent the possible escape of arthropods into the environment. Preventively, the area is equipped with adequate traps. Keep in mind that these actions may not inactivate the pathogenic micro-organism in question, hence recaptured arthropods should be collected and disposed of as infectious waste according to applicable waste law(s).

Points of attention:

- *In an emergency situation (large and uncontrolled release of arthropods), application of an insecticide or pesticide may be necessary. Careful consideration should be given to the impact of the insecticides or pesticides used on the arthropods housed elsewhere in the facility due to the necessary downtime of the room before experimenting again with arthropods.*
- *It is recommended to frequently change the type of disinfectants and insecticide or pesticide to avoid resistance development.*

Additional work practices specific to arthropods				
- Physical containment (closed system)	Recommended (one-layer containment)	Required (one-layer containment)*	Required (one-layer containment)*	Required (double-layer containment)
- Monitoring and controlling numbers of arthropods	Not required	Not required	Required	Required
- "Buddy principle" (or work in pairs)	Not required	Not required	Optional	Optional

The primary containment for housing arthropods should be appropriate depending on the arthropod's characteristics (flying, crawling, jumping, aquatic…), their requirements (feeding, lighting, temperature, humidity…) and to the combined use of live plants. It should allow for easy removal and reintroduction of the arthropods without risk of escape.

* The containment stringency should be proportional to the risk (severity and likelihood) for the human health and the environment. In G2/G2Q where determined by risk assessment, escape prevention must be additionally ensured by a double layer containment, e.g. in case the control of escapees is not possible by counting (because the number of arthropods is too high for instance).

Points of attention:

- *Mesh, when used, should be of an adequate size and robust.*
- *Cages should be visually examined regularly for structural damage and mesh integrity.*
- *The climatic chamber, if used as primary containment, should be equipped with a glass door or a mesh door to allow for visual inspection without having to open doors. The climatic chamber may create waste water and infectious aerosols which must be contained in an appropriate way (PPEs, HEPA filtered ventilation, waste management, decontamination management…).*
- *It may be necessary to foresee different types of containment for arthropods at different developmental stages.*

- A way of arthropods control is tracking the numbers of arthropods housed within a primary containment. Accurate counting and recording at each stage of handling is preferred until final disposal of the arthropods to detect any losses. If the numbers are no longer correct and the missing arthropods cannot be found and capture immediately, emergency procedures must be activated to mitigate the possible biological risk caused by the escape.
Counting is facilitated if the number of arthropods used is limited. Counting the number of insects for rapidly proliferating insects (such as aphids) is not feasible. An alternative is monitoring the number of infected plants.
In case the control of escapees is not possible by counting (because the number of arthropods is too high for instance), multiple physical barriers between arthropods and the greenhouse environment can be used.

- A **“**buddy system” or working in pairs consists of a collaboration between two trained workers to carry out delicate operations. It can reduce the risk of certain tasks and errors with arthropods by providing direct practical support for the study or seeking assistance in the event of an emergency.

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References

[1] Belgian regulation on the contained use of GMOs and/or pathogens: https://www.biosafety.be/content/contained-use-gmos-and-pathogens
[2] American Committee of Medical Entomology, American Society of Tropical Medicine and Hygiene; Arthropod Containment Guidelines, Version 3.2, vector-borne and zoonotic diseases, Volume 19, number 3, 2019
[3] Zach N. Adelman, David Pledger and Kevin M. Myles; Developing standard operating procedures for gene drive research in disease vector mosquitoes, Pathogens and Global Health, 2017 VOL. 111, NO. 8, 436–447; https://doi.org/10.1080/20477724.2018.1424514
[4] Mark Q. Benedict, Austin Burt, Margareth L. Capurro,, Paul De Barro, Alfred M. Handler, Keith R. Hayes, John M. Marshall, Walter J. Tabachnick, and Zach N. Adelman, Recommendations for Laboratory Containment and Management of Gene Drive Systems in Arthropods. Vector-borne and zoonotic diseases Volume 18, Number 1, 2018; DOI: 10.1089/vbz.2017.2121
[5] Infravec2 Horizon 2020; Guidelines for the design and operation of containment level 2 and 3 insectaries, Version 1, 2018. https://infravec2.eu/project-documents/
[6] Arthropod Containment Guidelines, Version 3.2; American Committee of Medical Entomology; American Society of Tropical Medicine and Hygiene 2019.
[7] Cécile J. B. van der Vlugt, David D. Brown, Kathleen Lehmann, Amaya Leunda, and Nicolas Willemarck. A Framework for the Risk Assessment and Management of Gene Drive Technology in Contained Use. Applied biosafety, 2018 Vol 23 (1) 25-31; DOI: 10.1177/1535676018755117
[8] Regulation (EU) 2016/2031 of the European Parliament of the Council of 26 October 2016 on protective measures against pests of plants : https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32016R2031
[9] Commission Delegated Regulation (EU) 2019/1702 of 1 August 2019 supplementing Regulation (EU) 2016/2031 of the European Parliament and of the Council by establishing the list of priority pests: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32019R1702
[10] Regulated plant pests in Belgium, Federal Agency for the Safety of the Food Chain (FASFC) : AFSCA - Organismes nuisibles réglementés des végétaux (favv-afsca.be) ; FAVV - Gereglementeerde schadelijke organismen bij planten (favv-afsca.be)
[11] EPPO activities on plant quarantine: https://www.eppo.int/ACTIVITIES/quarantine_activities
[12] Kairo G, Pioz M, Tchamitchian S, Pelissier M, Brunet JL, Belzunces LP. Efficiency of an air curtain as an anti-insect barrier: the honey bee as a model insect. Pest Manag Sci. 2018 Dec;74(12):2707-2715. doi: 10.1002/ps.5090. Epub 2018 Jul 22. PMID: 29808535.