

## Containment Management for Research and Development of GM Crops

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### Abstract

For GM research, containment targets both the prevention/ limitation of dispersal and impact of biological material outside of the contained facility as well as the protection of staff working in the facility. In spite of differences in regulatory approaches, most countries require organisations to identify their contained use activities, to conduct a risk assessment and to implement appropriate risk management measures. This also involves the assignment of responsibilities in the process and sometimes obtaining permits. When performing the risk assessment, both the known hazards (e.g. when dealing with a pathogen or pest) and uncertainty (e.g. when working with GM plants in the early stages of development) must be considered. Following the identification of possible hazards and potential risk mechanisms, control measures can be identified that prevent potential negative impacts. On this basis, a biosafety level (or category) is assigned which provides a combination of administrative controls, work practices and procedures, equipment, and facility elements required to achieve a designated degree of containment. Containment measures can be fine-tuned due to the availability of different options. Understanding how plant breeding and plant pathogen projects with non-GMOs are safely conducted provides valuable insight. Risk assessments of GM crop activities in glasshouses indicate that these projects can be safely conducted in the lowest level of containment. This can also include screenhouses for the development stages of some crops and plants. Activities involving GM microorganisms, pathogens and/or pests require a wider range - and usually a higher biosafety level - of containment measures. Nevertheless, the same approach of risk assessment-based identification of appropriate measures can be applied. This should enable scientists and regulators to ensure that early phases of GMO research can be conducted in a safe way without facing a disproportionate management burden.

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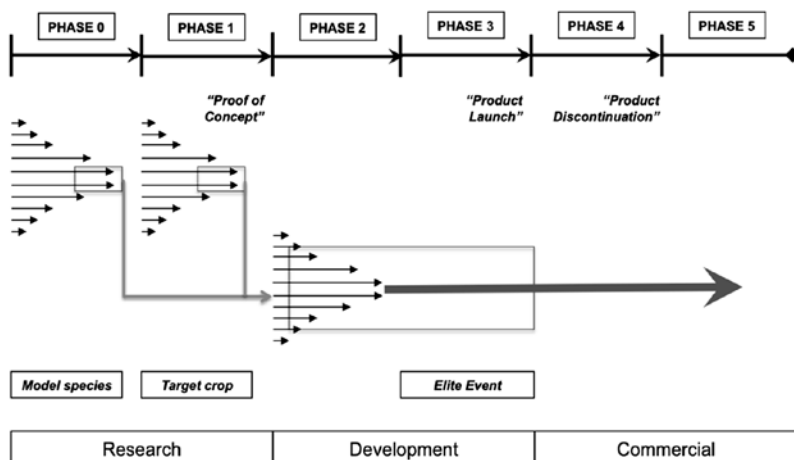
*Keywords: biosafety, containment, glasshouse, screenhouse, genetically modified crop, GMO.*

### **Riassunto**

Nell'ambito della ricerca sugli organismi geneticamente modificati (GM), il contenimento si focalizza sia sulla prevenzione/limitazione della dispersione, sull'impatto del materiale biologico al di fuori delle strutture di impianto confinato, sia sulla protezione del personale che lavora in tali strutture. Nonostante le differenze negli approcci normativi, la maggior parte dei paesi richiede alle organizzazioni di individuare le loro attività di impiego confinato, di effettuare una valutazione del rischio e di attuare adeguate misure di gestione. Ciò implica anche l'assegnazione delle responsabilità all'interno del processo e a volte l'ottenimento di autorizzazioni. Quando si esegue la valutazione del rischio, devono essere considerati sia i rischi noti (ad esempio, quando si abbia a che fare con un agente patogeno o parassita) sia le situazioni di incertezza (ad esempio, quando si lavori con piante GM nei primi stadi di sviluppo). Dopo il riconoscimento di eventuali pericoli e dei potenziali meccanismi di rischio, possono essere identificate le misure di controllo atte a prevenire i potenziali effetti negativi. Su queste basi, viene assegnato un livello di biosicurezza (o categoria) che prevede un insieme di controlli amministrativi, pratiche e procedure di lavoro, attrezzature, e tutti gli elementi necessari per raggiungere il livello designato di contenimento. Le misure di contenimento possono essere migliorate grazie alla disponibilità di diverse alternative. Capire quanto i progetti di miglioramento genetico delle piante e quelli sui patogeni delle piante nel caso di non-OGM siano condotte in modo sicuro fornisce informazioni importanti. La valutazione del rischio di colture GM in serra indica che questi progetti possono essere condotti in modo sicuro nel più basso livello di contenimento. Ciò anche durante le fasi di sviluppo di alcune piante coltivate e non. Le attività che coinvolgono microrganismi GM, patogeni e/o parassiti, richiedono una gamma più ampia di misure di contenimento e di solito un livello di biosicurezza più elevato. Tuttavia, può essere applicato lo stesso approccio di valutazione del rischio basato sulla identificazione di misure appropriate. Questo dovrebbe permettere agli scienziati e alle autorità competenti per la regolamentazione, di garantire che le fasi iniziali della ricerca sugli OGM possano essere condotte in modo sicuro senza dover gestire un carico di spesa sproporzionato.

## 1. INTRODUCTION

The first phase (Phase 0) of the life cycle of a biotechnology crop covers basic research to identify new functions and techniques in model species (Figure 1). Phase 1 then establishes 'proof of concept' in the target crop. In Phase 2, actual product development is initiated with carefully designed transformations and elite event selection. Development continues in Phase 3 by collecting information and obtaining approvals required for product launch. In subsequent phases, the product is on the market. Following the discontinuation decision at the end of Phase 4, the product is gradually removed. Although the phases are shown as being equal in length, in reality this will not be the case: for an annual crop Phase 1 to 3 may require 7 to 10 years and a successful product may then be available for several decades.



**Figure 1. Schematic overview of the life cycle of a plant biotechnology product** (Boxes indicate activities in confined field trials). Note: all phases do not need to be included in each project.

So far, much of the international capacity building effort for handling genetically modified (GM) crop has focussed on the later phases of

development projects (mainly phase 2 and phase 3) covering confined field trials, large-scale releases and market introduction. Given the potential concerns for human health and the environment, this focus has allowed countries to evaluate the applicability of GM crops that have already been developed and reviewed abroad, to gain experience with risk assessment and management processes, and to put in place accompanying measures deemed necessary for market introduction. Preparatory activities in containment (including the laboratory, glasshouse and screenhouse) were mostly considered on the basis of subsequent project steps and the safety features of these were in many cases addressed based on a standardised approach.<sup>1</sup>

These first applications have enabled the establishment of the later part of the product pipeline (e.g. by establishing a framework for conducting confined field trials). While many countries will likely continue to access improved GM plants in this late phase, more and more research organisations are establishing local capacity in plant biotechnology research and early development. This important development marks the success of technology transfer, which enables local scientists to perform research and develop crops and traits that are relevant for, and fully adapted to, local, sometimes niche, markets. Scientists and regulators now face the challenge to broaden the framework to ensure that the early research phases can be conducted in a safe way without imposing a disproportionate management burden.

This paper focuses on the containment of GM plants once they are removed from *in vitro* culture. Performing plant transformation and *in vitro* selection of transformants requires containment and presents specific challenges and options, which will not be covered here. While the well-established sequence of 1) molecular work in the laboratory, 2) plant transformation, again in the laboratory, 3) transfer of transformants to the glasshouse, 4) subsequent generations of transformants in the glasshouse and screenhouse remains valid, several variants are being explored (e.g. flower dip transformation, agro-inoculation, working with viral vectors...). These require a more thorough understanding of appropriate containment measures.

<sup>1</sup> The ICGEB Collection of Biosafety Reviews is no exception as this is the first review addressing containment since the series began in 2003.

**Table 1. Overview of biological material requiring biosafety considerations that may be involved of contained use of plants** (based on NIH, 2013)

<b>Biological material</b>	<b>Scope</b>	<b>Consideration</b>
<b>Plants</b>	<ul style="list-style-type: none"> <li>• Including mosses, liverworts, macroscopic algae, and vascular plants, including terrestrial crop, forest, weed and ornamental species.</li> </ul>	<ul style="list-style-type: none"> <li>• If GM</li> <li>• If considered invasive species</li> </ul>
<b>Plant-associated microorganisms</b>	<ul style="list-style-type: none"> <li>• Including those known to cause plant disease, such as viroids, virusoids, viruses, bacteria, and fungi, as well as protozoa and microorganisms that have a benign or beneficial association with plants, such as certain <i>Rhizobium</i> species,</li> <li>• Microorganisms known to cause plant diseases,</li> <li>• Microorganisms that are modified to foster an association with plants,</li> <li>• Microorganisms modified to transform the plant in a stable or transient way,</li> <li>• Microorganisms associated with plant-associated small animals (e.g., pathogens or symbionts).</li> </ul>	<ul style="list-style-type: none"> <li>• If GM</li> <li>• If considered a transformation vector</li> <li>• If considered disease</li> </ul>
<b>Plant associated small animals</b>	<ul style="list-style-type: none"> <li>• Including those arthropods that are: (1) in obligate association with plants; (2) plant pests; (3) plant pollinators, or; (4) transmit plant disease agents,</li> <li>• Other small animals such as nematodes for which tests of biological properties necessitate the use of plants</li> </ul>	<ul style="list-style-type: none"> <li>• If GM</li> <li>• If considered pest or disease</li> </ul>

In addition, the GM plant should not be considered in isolation. For testing certain traits, experiments involving challenges with pathogens or pest organisms may be required. The challenging organism may impose other

safety issues and thereby necessitate a different type and higher level of containment. To illustrate this, Table 1 provides an overview of the type of biological material that may be within the scope of a contained use and refers to particular considerations for biosafety measures.

## 2. DEFINING CONTAINMENT

“Containment” is usually understood to be associated with an act to prevent uncontrolled dispersal. “Contained use” can be interpreted as a particular activity accompanied by actions and measures taken to prevent spreading. Yet, over time the concept has evolved, leading to different perspectives. The following overview provides a few examples:

- As a consequence of the first call for precaution when dealing with recombinant organisms in the early '70s, the USA National Institutes of Health (NIH) developed guidelines, which have been regularly updated. The current guidelines (NIH, 2013) provide indications on how to achieve containment and defines the purpose of containment is to:
  1. Avoid unintentional transmission of recombinant DNA-containing plant genomes or release of recombinant DNA-derived organisms associated with plants;
  2. Minimise the possibility of unanticipated deleterious effects on organisms and ecosystems outside the experimental facility;
  3. Avoid the inadvertent spread of a serious plant pathogen from a glasshouse to a local agricultural crop, and;
  4. Avoid the unintentional introduction and establishment of an organism in a new ecosystem.
- In a milestone publication, the Organisation of Economic Considerations and Development (OECD, 1986) elaborated the purpose of containment for large-scale industrial productions was: to reduce exposure of workers and other persons; to prevent release of potentially hazardous agents into the outside environment, and; to protect the product. They outlined the means by which these objectives could be achieved either by “biological containment” through exploiting natural barriers which limit an organism’s ability to survive and/or transfer genetic information into specific environments or by “physical containment”.
- When the EU introduced its GMO-specific environmental protection

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framework in 1990, activities were divided into either “contained use” or “deliberate release”, each being covered by a specific Directive. In this context, “contained use”<sup>2</sup> means any activity in which organisms are genetically modified or in which such GMOs are cultured, stored, transported, destroyed, disposed of or used in any other way, and for which specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment. Likewise, “deliberate release” (EU, 2001) is defined as “any intentional introduction into the environment of a GMO or a combination of GMOs for which no specific containment measures are used to limit their contact with, and to provide a high level of safety for, the general population and the environment.”

- Australian regulations follow a similar dichotomy in defining “dealings” with GMOs in the Gene Technology Act (Australian Government, 2000) that include: “Dealings Not involving an Intentional Release” (DNIR) into the environment, i.e. dealings with GMOs in contained facilities, and; “Dealings involving an Intentional Release” (DIR) of GMOs into the Australian environment, i.e. dealings with GMOs which take place outside of containment facilities. Examples of facilities are provided and include buildings or parts of a building, laboratories and glasshouses.
- The Cartagena Protocol on Biosafety to the Convention on Biological Diversity (Secretariat of the Convention on Biological Diversity, 2000) defines “contained” use as “any operation, undertaken within a facility, installation or other physical structure, which involves living modified organisms that are controlled by specific measures that effectively limit their contact with, and their impact on, the external environment”.

From this selection of resource publications, common elements describing containment include:

- Combined biological and physical features,
- Some type of facility or physical structure as a basis,
- An intention to protect the population, including staff, and the environment,
- The prevention/limiting of dispersal and impact of material outside of containment, and

<sup>2</sup> Whereas the European Directive on “contained use” covers activities with GM microorganisms, most EU Member States extended the scope to include all GMOs.

- The understanding that any release would be non-intentional (i.e., should be considered as an incident).

For activities in laboratories, culture rooms (e.g. for *in vitro* cultivation of transformants) and growth rooms<sup>3</sup>, it can easily be demonstrated that these fulfil the conditions for contained use. Most glasshouses<sup>4</sup> and many screenhouses<sup>5</sup> also fulfil containment criteria; yet, there may be types that are more likely to be considered “deliberate release” (e.g. some amateur-garden-type greenhouses may not provide suitable containment). On the contrary, certain confined field trials involve a combination of confinement measures that may seem to be more in line with the definition of containment, rather than release. Therefore, although the definitions of containment and release are mutually exclusive, there is a “grey” area for which a more detailed specification is required to determine if contained use is still applicable.

### 3. LEGAL FRAMEWORK

Some regulatory approaches (e.g. in Europe and Australia) present a specific set of rules for dealing with the contained use of GMOs. This allows the tailoring of the risk assessment and management to the specific situation and protective environment that is offered when working in containment. Other legal approaches have left the contained use of GM plants outside of regulatory scrutiny. These systems rather focus on preventing unintentional release, on providing other forms of guidance (e.g. guidelines) for the safe conduct of such activities and on scrutinising only certain contained uses e.g. of potentially hazardous production systems. Both approaches result in contained use activities that are, compared to deliberate release, relatively straightforward to implement and thereby conducive for early research and development.

Driven primarily by Article 8(g) of the Convention on Biological Diversity (Secretariat of the Convention on Biological Diversity, 1992), many

<sup>3</sup> A room with a floor and non-transparent walls equipped for growing plants in a controlled and protected environment.

<sup>4</sup> A structure with a floor and transparent walls and roof designed and used principally for growing plants in a controlled and protected environment.

<sup>5</sup> A structure with a roof, floor and screened walls, designed and used principally for growing plants in a protected environment.



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governments have, or are in the process of establishing, regulatory frameworks for the safe handling of GMOs. National initiatives have been boosted by international agreements (e.g. the Cartagena Protocol on Biosafety [Secretariat of the Convention on Biological Diversity, 2000]) and capacity building programmes (e.g. by the United Nations Environment Programme [UNEP]-Global Environment Fund [GEF]). As these initiatives are mainly driven by concerns of deliberate releases and market introductions, the specifics of contained use are sometimes lost. Without this differentiation, the same procedures can prevail for any type of use, imposing an unjustified burden on local R & D initiatives.

Furthermore, the Cartagena Protocol on Biosafety does not provide a good basis for establishing contained use provisions. The Protocol requires Parties to make decisions on import of LMOs for intentional introduction into the environment in accordance with scientifically sound risk assessments. With this purpose to the fore, it sets out general principles, methodological steps, and points to consider in the conduct of risk assessment. The specific aspects of contained use are not covered and are explicitly left to be determined by the national authority. As will be explained below, while the principles of risk assessment may be the same, the focus on containment measures differentiates contained use from deliberate release. This underlies the fundamental dichotomy of the two approaches as implemented in most regulatory systems.

When planning to perform activities with organisms that may have an adverse effect on plant health, agronomic production, the local environment and/or economy, other agreements may also prevail. For example, the International Plant Protection Convention (IPPC; [www.ippc.int](http://www.ippc.int)) is a treaty concerned with preventing the introduction and spread of pests to plants and plant products. The IPPC has developed phytosanitary guidelines and serves as a reporting centre as well as an information source. Furthermore, the IPPC operates in close collaboration with regional plant protection organisations.

In spite of the differences and basis for the regulatory approach, a comparison of the main legal systems and guidelines reveals certain common aspects. The first and foremost is the requirement for any organisation to identify its activities that are to be considered as contained

use, to conduct a risk assessment and to implement appropriate risk management measures (these points will be discussed in more detail below). This also requires the assignment of responsibilities throughout the regulatory process and may involve obtaining permits.

### 3.1. Responsibilities

Biosafety/containment-associated tasks must be identified and these may lead to the assignment of functions in an organisation, as listed in Table 2. While the assignment will differ between organisations, and not all of these functions will be required in every case, the tasks need to be fulfilled and should therefore be clearly allocated.

Although these tasks and functions provide the backbone for a biosafety/containment programme, containment will be managed mainly by a diversity of staff members. Experience shows that most accidents are not due to infrastructure or equipment failures, but can be attributed to human factors. Of paramount importance, all staff involved must be trained for the conditions and risks appropriate for the assigned biosafety level. In addition, it is important that an atmosphere of continuous improvement is created in which people voluntarily report problems and areas for improvement. Supervisors should understand the importance of attitudes and human factors in their own efforts to control containment. Some observations that may be of help to supervisors are:

- Over-burdening, poorly-understood rules can lead to intentional violations or negligence,
- The routine nature of procedures provides the most opportunities for making mistakes,
- Work should occur at a 'normal' rate of speed, with extra care and supervision inputted at peak moments. Similarly, involving staff members who are not in an optimal condition (are too tired, too stressed, etc.) creates a greater potential for accidents,
- Working in a well-organised and uncrowded facility minimises the number and nature of incidents,
- Staff need to be aware of what is considered an incident, how to report it, and the broader benefits of reporting.

**Table 2. Responsibilities and tasks related to biosafety management**

<b>Management</b>	<p>It is management's ultimate responsibility to ensure that the working environment is safe. Duties include:</p> <ul style="list-style-type: none"><li>• Undertaking a risk assessment covering both human health and safety and environmental safety,</li><li>• Appointing an Institutional Biosafety Committee (IBC) and/or Bio(logical) Safety Officer (BSO) to advise/assist on risk assessment,</li><li>• Ensuring that adequate containment facilities and procedures are in place to control any risks to workers and the environment,</li><li>• Formulating and implementing local rules, procedures, etc.,</li><li>• Formulating and implementing emergency plans and procedures,</li><li>• Maintaining and testing containment equipment at regular intervals,</li><li>• Where necessary, monitoring for the presence of viable GMOs outside of containment,</li><li>• Providing training commensurate with the level of risk.</li></ul>
<b>Principal Investigator (PI)</b>	<p>The PI is ultimately responsible for the research project and for ensuring compliance with biosafety standards. This includes:</p> <ul style="list-style-type: none"><li>• Performing the initial risk assessment and recommending containment measures for the project,</li><li>• Complying with permit and shipping requirements,</li><li>• Developing the necessary containment protocols,</li><li>• Ensuring the integrity of biological and physical containment,</li><li>• Providing staff with protocols describing potential biohazards and necessary precautions,</li><li>• Instructing and training staff in: (i) the practices and techniques required to ensure safety, and (ii) the procedures for dealing with accidents,</li><li>• Supervising staff to ensure that the required safety practices and techniques are employed,</li><li>• Correcting work errors and conditions that may result in compromising the containment of GM materials,</li><li>• Adhering to approved emergency plans for handling any accidental spills, personnel contamination and breach of containment.</li></ul>

<b>Institutional Biosafety Committee (IBC)</b>	<p>The committee is preferably composed of members that are familiar with biosafety issues and have a demonstrated commitment to the surrounding community, especially pertaining to human and environmental protection. Tasks may include:</p> <ul style="list-style-type: none"> <li>• Acting as a contact point for authorities,</li> <li>• Advising on the risk assessment or any activity that requires biosafety considerations,</li> <li>• Reviewing research programmes or proposals and evaluating the adequacy of the containment measures proposed by the PI,</li> <li>• Providing local review and oversight of activities with GMOs by evaluating facilities, procedures, and the expertise of personnel involved in the research,</li> <li>• Ensuring that research with GMOs is in compliance with the applicable regulations and guidelines,</li> <li>• Adopting emergency plans for responding to any breach of containment.</li> </ul>
<b>Bio(logical) Safety Officer (BSO)</b>	<p>This person assists and advises management and the PI on biosafety matters. S/he can be mandated to of the responsibility of certain management tasks to secure the biosafety of the installation. Typical tasks include:</p> <ul style="list-style-type: none"> <li>• Supervising risk assessments,</li> <li>• Coordinating notifications and applications related to activities with GMOs,</li> <li>• Ensuring maintenance and control of equipment,</li> <li>• Monitoring waste treatment as well as disinfection of rooms, equipment, etc,</li> <li>• Controlling storage and transport of GMOs and/or pathogens,</li> <li>• Identifying training needs,</li> <li>• Organising and participating in internal inspections and audits,</li> <li>• Evaluating actions in the case of accident or release.</li> </ul>

Further, the competent authorities have also to fulfil certain tasks (see Table 3 for examples), and can be assisted by advisory committees to evaluate the scientific and technical aspects of specific applications.

**Table 3. Responsibilities and tasks of competent authorities responsible for contained use** (based on UK Health and Safety Executive structure, <http://www.hse.gov.uk/biosafety/gmo/hseandgmos.htm>)

<b>Policy team</b>	<ul style="list-style-type: none"> <li>• Negotiate and implement international laws and conventions on biological agents and GMOs,</li> <li>• Develop national legislation,</li> <li>• Ensure that the regulatory regime covering GMO/ biological agents is in line with overarching government policies.</li> </ul>
<b>Notification team</b>	<ul style="list-style-type: none"> <li>• Manage the notification scheme for premises and contained use work with GMOs,</li> <li>• Undertake the administrative procedures involved in the handling of notifications under the contained use regulations.</li> </ul>
<b>Inspector team</b>	<ul style="list-style-type: none"> <li>• Provide advice and guidance to centres working in containment with GMOs.</li> <li>• Undertake targeted inspections of premises carrying out contained use.</li> </ul>

### 3.2. Permits

Depending on the regulatory approach, a permit may be required before conducting activities with GM plants in containment. In those countries that have opted to rely on guidelines, this is typically not required and it is up to the user to ensure proper containment. Other countries (e.g. EU and Australia) have established a double permit system:

- Facility permits certifying the containment features of a facility and indicating the suitability of the facility to provide a specific containment level (see below). Such a permit or license is typically provided for a longer period (several years) and/or until the facility is changed in a way that impacts the containment features.
- Activity permits are provided for individual activities, projects, etc. Based on the risk assessment for the individual activity, a risk level is determined and adequate containment features are identified. The permit then establishes the link with the facility permit, confirming that the activity can be conducted under the specific conditions.

In a double permit system, the first use typically involves both a facility and at least one activity permit. Subsequent activities can be handled based on the initial facility permit.

Many legal systems have simplified administrative procedures for low risk contained use activities. As an example, Table 4 provides some procedural aspects of different contained use notifications in the EU. Differentiation is determined by the risk class of the activity and whether it concerns a first of multiple uses. In the case of operations involving high risk, the consent of the competent authority must be given before the start of the activity. Lower risk class activities are allowed to proceed with a minimal administrative coverage (note: even in the case of minimal administration, e.g. risk class 1 use, a risk assessment must be established and kept available for inspection by the competent authority).

### **3.3. Public involvement**

As procedures differ, the level of public information and involvement is also very different depending on the regulatory approach. Those members of the public and stakeholders that may be directly affected in an accident, especially for higher risk level facilities, can be involved in the decision process. Conversely, much of the information concerning contained activities during the early R&D stages may be considered as confidential in order to protect the competitive position on the developer.

Regulators try to find a balance between securing the interest of an R&D organisation and the call of the public and stakeholders for access to information. This can be achieved by addressing the type of activities and the protective measures in place at a facility.

## **4. RISK ASSESSMENT**

Gray (2012) provides a practitioner's approach to the risk assessment of GM crops in which the identification of protection goals is an initial step in determining the problem context. Although this process has been mainly formulated for GMO introductions in the environment, it is also relevant for contained use. Whilst the predictive evaluation of any possible impact of introducing GMOs in the environment is emphasised, for contained use the focus is on preventing any introduction and thereby avoiding the need for a detailed impact assessment. In a conceptual model for risk assessment,

contained use is implemented in order to prevent certain steps from occurring and thereby reduce the likelihood of specific risk scenarios from being realised.

**Table 4.** Example of differentiated administrative procedures depending risk class of the activity and whether it concerns a first or subsequent use (Subs) (based on EU, 2009)

	Risk Class 1		Risk Class 2		Risk Class 3		Risk Class 4	
	First	Subs	First	Subs	First	Subs	First	Subs
<b>Risk assessment</b>	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
<b>Notification to competent authority</b>	Yes	- <sup>1</sup>	Yes	Yes	Yes	Yes	Yes	Yes
<b>Proceed with the contained use unless otherwise indicated by competent authority (number of days between notification and start of activity)</b>	1	-	45	1 <sup>2</sup>	-	-	-	-
<b>Formal consent by competent authority required (max. number of days for delivery of consent)</b>	-	-	-	-	90	45	90	45

<sup>1</sup> It is sufficient to keep the risk assessment available for competent authority.

<sup>2</sup> An option is provided to request a formal decision by the competent authority (delivered within max. 45 days)

Containment provides safeguards for activities for which the inherent risks are well known and high as well as when addressing risk scenarios that are highly hypothetical but for which the impact is potentially important.

Contained use is therefore an essential tool in the early phases of a project as it allows the project to proceed in the absence of detailed information on the relevance of certain risk scenarios. Whilst ensuring that a risk scenario cannot be realised, information can be generated that will eventually support the next step in development, i.e. confined field trials. This is fully in line with the precautionary principle, requiring a risk assessment to be conducted and proportionate measures to be implemented even if there is insufficient proof that an important risk will materialise.

Conversely, the hazard is known in the case of activities with plant-associated organisms that are known to cause unwanted effects such as diseases and pests. Here the containment measures are designed to ensure that there is no impact beyond the experimental setting.

Whilst both aspects of containment are based on the same measures, it is important to keep in mind the distinction between precaution, dealing mostly with uncertainty, and prevention, addressing known hazards. One of the distinctions is the ultimate purpose: known hazards will remain hazards and must be managed under all circumstances, whereas uncertainties can be further documented to allow a gradual reduction of protective measures (known as the “step-by-step approach”).

In all cases, a risk assessment will be the basis to determine the appropriate level of containment. There are several guidance documents on criteria to take into account. As an example, elements are provided in Table 5.

The plant species’ “weediness”, reproductive characteristics, and the presence or absence of sexually-compatible relatives in the surrounding environment are critical criteria considered in assigning a risk category to the GMO.

The hazards associated with the biological material can be realised via different mechanisms, which are partly determined by the type of organism and the development stage (e.g. vegetative material, survival structures,



pollen, spores, microorganisms, larvae, flying stages, etc.):

- Exposure of workers in the contained facility,
- Dissemination by humans,
- Dissemination by vector organisms (e.g. insects, rodents, birds, mammals),
- Dissemination by air,
- Dissemination by soil,
- Dissemination by run-off water,
- Dissemination via tools and equipment,
- Dissemination by waste.

Based on the identification of possible hazards and potential mechanisms, control measures can be identified that prevent specific mechanisms from materialising.

**Table 5. Considerations for determining risks associated with activities involving GM plants and plant-associated GM microorganisms** (based on SACGM, 2007)

Risk assessment	Activities with GM plants	Activities with plant-associated GM microorganisms (GMM)
<b>For the environment</b>	<ul style="list-style-type: none"> <li>• Hazards associated with the inserted gene/element (ability to cause harm to plants, animals or beneficial microorganisms; transencapsidation, recombination, satellite systems, synergistic effects),</li> <li>• Transfer of harmful sequences between organisms,</li> <li>• Phenotypic and genetic stability,</li> <li>• Capacity to survive, establish and disseminate.</li> </ul>	<ul style="list-style-type: none"> <li>• Hazards associated with the recipient organism (e.g. when it concerns a pathogen),</li> <li>• Hazards associated with inserted genes,</li> <li>• Hazards arising from the alteration of existing traits (survivability, stability, infectivity, pathogenicity, effects on host plant defence, tropism, host range, transmissibility),</li> <li>• Transfer of harmful sequences between organisms,</li> <li>• Phenotypic and genetic stability,</li> <li>• Ability of the GMM to become established</li> </ul>
<b>For human health</b>	<ul style="list-style-type: none"> <li>• Modifications that affect allergenicity or toxicity of a plant.</li> </ul>	<ul style="list-style-type: none"> <li>• Secretion of toxic products,</li> <li>• Modifications that alter properties of an infected plant.</li> </ul>

On this basis, a biosafety level is assigned which provides a combination of required administrative controls, work practices and procedures, equipment and facility features. The levels of containment range from the lowest biosafety level 1 to the highest at level 4<sup>6</sup>.

Adair & Irwin (2008) summarised criteria for assigning biosafety levels that are required for specific activities with plants and provided some examples (see Table 6). The table shows that as the potential risk to the environment increases, increasingly stringent requirements for containment are indicated. When applicable, physical containment requirements may however be eased with the addition of biological containment measures.

According to the NIH Guidelines (NIH, 2013), BL4-P containment is recommended only for experiments with readily transmissible exotic infectious agents whether transgenic or not, such as air-borne fungi or viruses in the presence of their arthropod vectors, that are potentially serious pathogens of major crops. In most cases, the conventional glasshouses typically used in research stations can be used or modified for GM plant experiments, if proper practices are developed and followed. Costly new constructions are generally not needed for levels BL1-P and BL2-P.

## 5. RISK MANAGEMENT

In the previous section, biosafety levels for activities with plants were introduced. Before addressing the containment provisions of these levels, it must be stressed that the containment measures are largely determined by the biological characteristics of the organism that needs to be contained. For example, if there are no sexually-compatible species in the area susceptible to pollen flow, then the need for blocking pollen dispersal is very much reduced. Similarly, if the environment of the glasshouse/screenhouse is not suitable for the establishment of the species, less stringent containment measures may still be adequate. This underlines the need to base the risk assessment on a thorough understanding of the biological material, combined with experience obtained in conventional breeding and agronomic research.

<sup>6</sup> Depending on the classification system, terminology and numbering may differ. In this paper the NIH classification is used. Additionally, facilities that have not been designed or intended for containment are not classified even if some containment features may be in place

**Table 6. Indications and examples of type of work in different containment biosafety levels** (based on Adair & Irwin, 2008)

	Indications for type of work	Example
<b>BL1-P</b>	<ul style="list-style-type: none"> <li>• For GMOs for which there is no evidence that they would be able to survive and spread in the environment,</li> <li>• If the GMO is accidentally released, it would not pose an environmental risk.</li> </ul>	<ul style="list-style-type: none"> <li>• Experiment designed to study GM potatoes containing cloned genes for insect resistance obtained from primitive potato cultivars,</li> <li>• GM common microorganisms that cannot spread rapidly and are not known to have any negative effects on either natural or managed ecosystems, such as <i>Rhizobium</i>.</li> </ul>
<b>BL2-P</b>	<ul style="list-style-type: none"> <li>• For GM plants and associated organisms, which, if released outside the glasshouse, could be viable in the surrounding environment but would have a negligible impact or could be readily managed,</li> <li>• For GM plants that may exhibit a new weedy characteristic or which may be capable of interbreeding with weeds or related species growing in the vicinity,</li> <li>• For GM plant research that uses the entire genome of an indigenous infectious agent or pathogen,</li> <li>• For GM plant-associated microorganisms that are either indigenous to the area and potentially harmful to the environment but manageable, or are exotic but have no potential for causing serious harm to managed or natural ecosystems,</li> <li>• For GM plant-associated insects or small animals if they pose no threat to managed or natural ecosystems.</li> </ul>	<ul style="list-style-type: none"> <li>• Glasshouse tests of GM sunflower containing wheat genes intended to confer resistance to the fungus <i>Sclerotinia</i> (in settings where sunflower is capable both of hybridising with wild relatives and becoming established as a volunteer weed).</li> </ul>

<b>BL3-P</b>	<ul style="list-style-type: none"> <li>• For GM plants, plant pathogens, or other organisms that have a recognised potential for significant detrimental impact on the environment,</li> <li>• For GM plants containing genes from an exotic infectious agent in which a complete functional genome of the infectious agent could possibly be reconstituted,</li> <li>• For GM plants or organisms that contain genes coding for vertebrate toxins,</li> <li>• For GM plants involved in PMP<sup>1</sup> and PMIC<sup>2</sup> production,</li> <li>• For GM microbial pathogens of insects or small animals that associate with plants, if the pathogen has the potential to cause harm to the local environment,</li> <li>• For non-GM plant research that involves exotic infectious agents capable of causing serious environmental harm.</li> </ul>	<ul style="list-style-type: none"> <li>• Testing citrus plants engineered to be resistant to Asiatic Bacterial Canker by infecting them with the disease pathogen in a location where, if released, the pathogen could devastate the commercial citrus crop,</li> <li>• Inoculating GM peanut plants containing fungal resistance genes with <i>Aspergillus flavus</i>, the organism responsible for producing the potent vertebrate mycotoxin aflatoxin.</li> </ul>
<b>BL4-P</b>	<ul style="list-style-type: none"> <li>• For certain exotic, readily-transmissible infectious agents that are potentially serious pathogens of major crops,</li> <li>• Human pathogens or vaccines made in plants that cause serious human illness.</li> </ul>	<ul style="list-style-type: none"> <li>• Test the ability of the maize streak virus coat protein to protect maize plants against infection by the virus, using its leafhopper vector, <i>Cicadulina</i> spp., in challenge inoculations. If the devastating virus is not endemic, but the leafhoppers capable of transmitting the virus are, then an experiment using both a serious pathogenic virus with its vector poses a significant risk should they escape the containment facility.</li> </ul>

<sup>1</sup> PMP – Plant Made Pharmaceutical; <sup>2</sup> PMIC – Plant Made Industrial Compounds.

Table 7 gives an overview of containment features that are indicated in different guidance notes. They are further discussed below. Although they are typically representative of specific containment levels, they should not be considered as an exhaustive or mandatory list. On the contrary, they reflect a multitude of technical options that can be considered in view of the specific scope of activities that are expected to be carried out in the facility.

**Table 7. Overview of containment measures as required for different containment levels**

	BL1-P	BL2-P	BL3-P	BL4-P
<b>Structure</b>				
• Permanent structure	☐	■	■	■
• Separated from other areas in the same building or in a separate building	-	-	■	■
• Rigid, reinforced glasshouse framing (typical aluminium or steel)	☐	☐	■	■
• Foundation of concrete, concrete block, brick or similar	☐	☐	■	■
• Glass or rigid thermoplastic	■	■	■	n.a.
• Sealed and break-resistant glazing material	-	-	■	■
• Openings to the exterior are screened with 30 mesh <sup>1</sup> or higher insect screens	■	■	n.a.	n.a.
• Concrete floors, with or without porous materials under benches	☐	☐	n.a.	n.a.
• Impervious floors	-	-	■	■
• Materials resistant to chemical disinfectants	-	-	■	■
• Containment compartments are sealable for fumigation	-	-	■	■
• Rigid, self-closing doors with excluders	-	-	■	■
• Key or system to restrict/ control access	-	-	■	■
• Entry via an airlock or a separate room with two interlocking doors	-	☐	☐	■
• Emergency doors without external hardware	-	-	■	■

• An observation window or alternative so that occupants can be seen	<input type="checkbox"/>	<input type="checkbox"/>	■	■
• Negative pressure relative to the pressure of the immediate surroundings	-	<input type="checkbox"/>	■	■
• HEPA <sup>2</sup> filtration of extracted air	-	-	■	■
• HEPA filtration of input air	-	-	-	■
• Control of contaminated run-off water	<input type="checkbox"/>	■	■	■
<b>Equipment</b>				
• Surfaces impervious to water and resistant to acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	■ (bench)	■ (bench)	■ (bench, floor)	■ (bench, floor, ceiling, walls)
• Microbiological safety cabinet/ enclosure	-	<input type="checkbox"/>	■	■
• Autoclave	■ (on site)	■ (in building)	■ (in contained area)	■ (in contained area/ passthrough)
• Contained area contains its own reserved equipment	-	-	■ (as reasonably practicable)	■
• Air curtains, light traps, etc. for trapping insects	-	-	■	■
<b>Personal protective equipment</b>				
• Suitable protective clothing	■	■	■	■
• Specific footwear reserved for contained area	-	-	■ (optional)	■
• Complete change of cloths	-	-	-	■
<b>Practices</b>				
• Written records of staff training	-	<input type="checkbox"/>	■	■
• Access restricted to authorised personnel only	-	■	■	■
• Entry log book	-	-	-	■
• Biohazard sign on access doors	-	■	■	■
• Indication of work in progress and contact details of staff responsible	-	■	■	■

• Hand washing	■	■	■	■
• Shower	-	-	□	■
• Safe storage of biological material	□	■	■	■
• Specific measures to control aerosol dissemination	-	■ (minimise)	■ (prevent)	■ (prevent)
• Effective control of vectors such as insects, rodents, arthropods which could disseminate the material	■	■	■	■
• Effective control of GMO pollen, seeds and other plant material which could disseminate	□	■ (minimise)	■ (prevent)	■ (prevent)
• Procedures for the transfer of living material between different facilities or rooms (if applicable), including record-taking	■ (minimise loss)	■ (minimise loss)	■ (prevent loss)	■ (prevent loss)
• Specified disinfection procedures	□	■	■	■
• Treatment of personal protective equipment before disposal or cleaning	-	-	□	■
• Inactivation of effluent from hand washing sinks and showers and similar effluents	-	-	□	■
• Inactivation of contaminated materials and waste by validated means	■	■	■	■
• Emergency procedures (unintentional release, exposure)	■	■	■	■
• Documented regular inspections	■	■	■	■
• Security provisions	-	-	■	■

- = not required;

□ = required where and to the extent that the risk assessment shows it is required;

■ = required.

Based on EU (2001) and SACGM (2007) as an example of national implementation in the UK; CFIA (2007), Adair & Irwin (2008), OGTR (2013), NIH (2013).

<sup>1</sup> "Mesh" is a commonly-used indication for sieve size. Mesh 30 corresponds to openings of 0.595 mm. The higher the number, the smaller the openings in the sieve through which particles can pass.

<sup>2</sup> HEPA - High efficiency particulate air.

The following section provides more detail on the above containment measures and options. Each of the points deserves a detailed study and the information is only intended to provide an indication of elements to consider. Readers are invited to seek further guidance in publications such as SACGM (2007), CFIA (2007), OGTR (2013) and NIH (2013). Large parts of

this section are based on Adair & Irwin (2008), which is considered a basic reference document for contained glasshouses.

### 5.1. Structure

The containment structure should be of a suitable design and construction and be appropriately maintained so as to withstand normal climatic conditions over the period of the activity. At the lowest containment level, this can be a non-permanent structure such as a basic polytunnel, although this may entail challenges to control against invertebrate and fungal vectors and to prevent pollen or seed dispersal. Consequently, most containment facilities will be permanent structures with walls, a roof and a floor. As these are permanent structures, the choice of site and design requires careful planning, both taking into account the available immediate and future resources. High-level containment facilities not only require important investments; recurrent support and maintenance costs should already be considered at planning stage.

Containment glasshouses should be situated and constructed considering factors that may damage the integrity of the structure, e.g. sufficiently distant from geographical features capable of impacting the structure such as roads (traffic accidents), surface water, flooding, violent storms, etc.. At the same time, the surrounding environment can be considered in relation to biological containment, e.g. the distance to compatible species will influence the need to limit pollen flow.

The design must take in to account that a containment glasshouse is seldom an entity unto itself. Supporting workspaces such as the headerhouse preparatory space, laboratories, growth chambers, incubators, tissue culture facilities, inoculation chambers, and maintenance areas are either located directly adjacent to, or within a reasonable distance, from the glasshouse. Finally, options for structural extension and adaptation can be anticipated by the use of a modular design that can be readily expanded.

Glasshouse structures are engineered to support cladding and other component loads, as well as to withstand minimal environmental stresses. The structural system consists of a primary roof, a secondary structure, columns, foundations, and cladding. High-level containment facilities require a reinforced, rigid frame for both security reasons and to



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accommodate the weight of mandated double-paned, break-resistant, sealed glass.

Different glazing materials have widely varying degrees of light transmission, longevity, flammability, selective measures of strength, and infiltration by air and water. Standard glasshouse glazing material will satisfy the requirements for BL1-P and BL2-P. Clear glass glazing is the most enduring and provides the greatest amount of natural light. Tempered, laminated, chemically strengthened, and/or multi-layer (double or triple) glass is preferred for high containment glasshouses. Also, sheets of rigid thermoplastic (polycarbonate or acrylic) can provide interesting solutions, usually with less weight and with excellent strength. In addition to the choice of glazing, proper sealing between the panes will contribute greatly to containment.

Gravel and soil beds can be used under benches in BL1-P glasshouses only if experimental material cannot move through these beds and leave the glasshouse. Concrete walkways are suggested for low-level containment. Regardless of the requirement, solid concrete flooring adequately sloped towards drains is preferred for all research glasshouses. For high-level containment, it is recommended installing impermeable floors that can withstand repeated applications of disinfectants. Properly sealed or coated (e.g. a slip resistant polymer floor system) concrete flooring is the most practical way to meet these and other high containment guidelines.

Filters or screens can be placed in the drains when working with small arthropods or plant pathogens or when movement of GM seeds has to be prevented. BL3-P and BL4-P facilities must have a system to collect all runoff. Runoff is then drained to a decontamination tank or treatment facility before release to a standard sewer or other disposal system.

Public access must be limited at all containment levels. Traditional cylinder door locks provide good security as long as strict key control is maintained. Electronic and electromagnetic systems utilising key cards combine highly restricted access with the possibility to log individual entries and exits. Regardless of containment needs, personal safety should never be compromised. For safety, emergency exit doors should be foreseen. They can be equipped with panic bars on the interior and should have no

exterior hardware (to avoid that they become an alternative entrance).

A self-closing, locking, steel door is preferable, though not always required. Standard lockable, hinged doors can be used for exterior and corridor entrances. Sliding doors are acceptable at BL1-P and BL2-P but do not seal tightly enough for higher containment levels. Both styles of doors can be fitted with locks to limit access. Extended-height kick plates can protect doors from structural damage caused by rolling carts. High containment facilities must have a double set of self-closing, locking, gasketed doors at entryways. Doors should fit tightly against the jamb and have a door excluder at the threshold.

An anteroom is recommended, if not required, for plant pathogen and arthropod work. A connected walkway, headerhouse, or preparatory room may serve as an anteroom in some situations, thus providing another layer of isolation between glasshouse material and the environment. A double-door entry system, with a dark anteroom sandwiched between the doors, aids in effective insect containment. In high containment facilities, the doors should be interlocked so that only one door can be opened at a time. A shower room or other controlled spaces may also act as an anteroom. Shoe baths and floor sticky mats should be placed at doorways and anterooms to trap materials that could be carried on footwear. For higher containment, shoe covers are often recommended.

Typical glasshouse heating systems include hot water radiation, steam radiation, infrared electric heaters, and forced air heating. The types of heating equipment used can be quite variable, including in-floor heating, finned-tube radiators, unit heaters, refrigeration coils, and bench heating. Air can be distributed through overhead tube assemblies or horizontal airflow fans. All of these systems are adequate for every containment level, if care is taken not to create spaces that are difficult to clean and disinfect.

Cooling a glasshouse is usually difficult and can be an important cost factor, so choosing the appropriate system is essential. Glasshouses can be cooled by:

- *Natural ventilation* - the most common and energy efficient method is simply to employ natural ventilation using motorised and/or manual

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- hinged vents located at the roof ridge and/or sidewall,
  - *Shade systems* - shade systems effectively provide an energy efficient form of passive cooling by reducing solar load,
  - *Exhaust fans* - louvered exhaust fans accelerate the exchange of warm air with the outside ambient air and are often combined with evaporative cooling pads,
  - *Evaporative methods* - high-pressure fog is an evaporative cooling system that can be used when the structure and climate permit. Fog droplets, ideally 20 microns or less in size, evaporate before landing, so free water is not deposited on plant leaves,
  - *Mechanical air conditioning* - i.e., air conditioning, is the only cooling option for a closed containment glasshouse. Mechanical cooling works by passing air over coils containing refrigerant, chilled water, or other chilled solution. When properly designed, this approach offers the most precise temperature control and uniform conditions but construction and operation costs are higher than other methods. Because mechanical cooling tends to dry the air, humidification is recommended.

Insect screening is recommended (BL1-P) and required (BL2-P) for all vent openings and motorised or gravity-driven exhaust fan louvers. Many types of screen size and composition are available. Screen mesh size should be gauged to the size and shape of the organisms and materials of interest. For arthropod containment, an 80-mesh, metallic screen can be adequate; a larger opening may be suited when working with noxious weeds or parasitic plants and a smaller opening size for pollen.

A range of measures is required to preserve containment when installing cooling systems. Generally, the structures for operating vents that pass through screen are fitted with brushes or flexible barriers to prevent rodents and other large pests from entering the glasshouse. Maintenance of cooling systems is required to sustain containment and includes ensuring that gaps around cooling pads are minimised or eliminated, fan louvers seal tightly when closed, and screens are clean. Dust accumulation on screens affect their efficiency therefore, as the screen opening size decreases, the need to clean screens by washing or vacuuming increases. The screens must be of a material mechanically strong enough to withstand any airflow load, remain undamaged with regular cleaning, and resist corrosion and penetration by animals, including invertebrates.

For containment purposes, screened side vents are recommended for BL1-P, and required for BL2-P. If evaporative cooling pads made of aspen fibre or corrugated cellulose are used on intake side vents or cooling units, screening can still be useful to prevent entry by insects.

Regardless of where screening is placed, airflow considerations are paramount because of temperature changes associated with reduced air movement. Airflow, cooling, and fan performance are significantly affected by the installation of any screen, especially when using finer mesh sizes.

At BL3-P or higher, glasshouse exhaust air must be filtered and the room held under negative pressure. Intake air is also routinely filtered to prevent the introduction of organisms from the environment into the enclosed space. Filter systems can be designed to trap pollen, spores, and other small particles. High efficiency particulate air (HEPA) filters can trap particles of 0.3 micron and larger diameter while allowing gases to transfer across the filter media. Pressure differences should be configured to direct airflow sequentially from the least hazardous or clean areas (held at positive or the least negative pressure) to the most contaminated areas (held at the most negative pressure) where the organisms of interest are generally handled. Research glasshouses, like any structure, cannot completely eliminate air infiltration. However, to preserve containment the infiltration rate should be controlled to allow an exchange rate of no more than one complete internal volume of air per hour.

In particular for high-level containment facilities, special care must be taken when installing and maintaining standard provisions, such as:

- *Lighting* - supplemental lights are commonly added to research glasshouses to aid plant growth and for general lighting. Lighting may impact containment due to the attraction of arthropods to light and may also be a problem if the design allows arthropods to persist inside the greenhouse.
- *Control and electrical systems* - the need for precise control and supplemental lighting in research glasshouses necessitates that high quality, large capacity electrical systems are installed. High containment facilities must have an even greater capacity because they require equipment redundancy and a backup method of electricity generation. High containment facilities also require that all electrical receptacles,

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outlets, and conduit are sealed to prevent the escape of GMOs, especially insect.

- *Piping* - heating, watering, and fertilising systems are typically piped into and throughout the glasshouse. For containment purposes, piped systems should be installed with a minimal number of intrusions.

High-level containment facilities may be required to be sealable for fumigation to facilitate decontamination in the event of a significant accidental release. Such sealability also protects humans outside of the facility from the potentially toxic effects of the fumigant. It is recognised, however, that fumigation against plant pathogens is not routine within plant growth facilities and may not even be possible in a glasshouse. Where the facility is not sealable and fumigation is not to be used, alternative means of decontamination must be documented and the materials of the structure must allow their implementation. For example, washing the facility down with a validated chemical disinfectant may be appropriate.

So far, we have focussed on glasshouse structural elements. Clearly, many of these containment requirements cannot be achieved in screenhouses. Consequently, screenhouses are only suitable for low containment-requiring activities (e.g. following the requirements for BL1-P or BL2-P level glasshouses, including floors). In such case, screenhouses may offer a low cost alternative to glasshouses when sited in an appropriate climate.

## **5.2. Equipment**

Many different types of benching can be used in research facilities. Bench material should be easily cleaned and resistant to acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use. Benches made of aluminium, galvanized steel, and certain plastics provide the longest wear, meet higher containment standards, and can be thoroughly cleaned, which benefits a pest control programme regardless of the research protocol. Wood is a poor choice because it may conceal pests. Benchtop materials that let water drain to the floor are most common because they permit drainage under plant containers and enhanced air circulation.

A large choice of trays, containers and pots is available. They can be single-use or reusable, in which case attention should be given to their decontamination and cleaning procedure. Growing plants directly in soil is usually not permitted in a contained glasshouse. When flowering of

the experimental plants is required, the isolation of pollen sources can be accomplished by bagging, netting, cages, etc. as for conventional breeding programmes. While researchers are more interested in installing these measures in order to avoid cross-pollination in the glasshouse, the measures also prevent pollen flow to the exterior. Similarly, measures can be taken (bagging, netting) at harvest to avoid seed loss. To avoid dispersal of small seeds e.g. from *Arabidopsis*, specialised growing apparatus such as the Aracon™ system ([www.arasystem.com/products/aracon](http://www.arasystem.com/products/aracon)) have been developed to both collect and contain seed.

Insect cages, when properly used, can increase the containment level of a particular experiment, as long as the factors listed above pertaining to screen characteristics and sizing are met. Researchers may fashion cages out of metal, wood, glass, or screen; however, effective commercial models are also available.

High-level containment facilities should contain their own equipment. This is to reduce the movement of experimental materials between different facilities and thereby reduce the likelihood of material dissemination. This requirement should be implemented so far as reasonably practicable, e.g. a unique and expensive piece of equipment can be shared in different activities. Irrespective, equipment should be thoroughly decontaminated before removal, repair or servicing.

For activities that may involve possible unwanted dispersal via air (e.g. working with spores of a fungal pathogen or with airborne pathogens), a biological safety cabinet (BSC) can be used. All exhaust air is HEPA-filtered as it exits the BSC, removing any harmful biological agents. This is in contrast to a laminar flow clean bench, which blows unfiltered exhaust air towards the user and is not safe for work with pathogenic agents. Importantly, most BSCs are not safe for use as fume hoods. If hazardous chemicals have to be manipulated at the same time as biological agents, special provisions have to be included.

The BSC offers protection against airborne particles. However, when working with arthropods it may be more efficient to use a screen cage as the manipulation space. If needed, it can be connected to a filtered negative pressure unit.

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To prevent the survival of organisms unintentionally transported outside of the glasshouse environment, experimental materials must be rendered biologically inactive (devitalised) before disposal. Devitalisation of plant material and soil should be completed before it leaves a glasshouse or laboratory and goes to a landfill. Plants and associated organisms can be inactivated by several methods:

- *High temperature* - the standard practice of heating materials to 85 - 100 °C for 30 minutes will kill almost all plant-associated organisms. When working with fungal, viral, or nematode plant pathogens, soil and other solid wastes should be treated to a minimum of 104 °C for three hours before disposal under permit. Steam forced into special carts or boxes has commonly been used in glasshouses for treating growing beds, pasteurising or sterilising media, and disinfecting containers. Sterilisation boxes with electric heating coils that deliver temperatures of 60 – 93 °C are also common. Material from smaller experiments can be inactivated by autoclaving all plants, plant parts, containers, and potting media. The recommendation is to autoclave materials at 103 kPa pressure and 121 °C for 15 – 180 minutes, depending on the type and state of the material being sterilised. The key requirement is that the system is validated to ensure sufficient steam penetration to the centre of the load for the required time period is achieved. At higher containment levels, the recommendation is to sterilise all materials leaving the greenhouse in an autoclave. A double-door, pass-through system for moving larger items in and out of containment is recommended. For liquids, a batch or pass-through type system that sterilises effluent before it enters the sewer is a good choice. Liquid effluent normally must be cooled before release. Incineration may also be used to destroy easily combustible, dry plant material; however, incineration must be used with caution since not all seeds are easily burned, e.g., cottonseed. Furthermore, incineration may conflict with local ordinances.
- *Low temperature (freezing)* - freezing is a common method for killing adult arthropods as most specimens die when kept overnight in a freezer. Some insects may require longer periods. However, the surrounding matrix can shield them from efficient cooling, so it is necessary to validate the method and to monitor the performance of the freezer.
- *Chemical treatment* - the standard practice of chemically treating glasshouse soil with methyl bromide, chloropicrin, and similar products

is being replaced by steam methods due to toxicity concerns. The chemosterilants ethylene oxide (EO) and vaporized hydrogen peroxide (VHP) are used in high containment facilities but require specialised application equipment.

- Containment facilities may use common disinfectants such as sodium hypochlorite, phenols, formaldehyde, glutaraldehyde, and alcohol. Chlorine as well as non-chlorine-based glasshouse disinfectant solutions that are safe for applicators and the environment are easily obtained from grower suppliers. The gravel under benches in BL2-P facilities can be decontaminated by, for example, treatment with 10 % sodium hypochlorite (household bleach) or similar solution. Periodic cleaning of all growing area surfaces with standard cleaning solutions or plain soap and water is highly recommended. Cleaning alone can be an effective decontamination method and also serves as preparation for VHP or any other surface sterilisation method.
- *Composting* - for large volumes, composting is an acceptable disposal treatment for experimental plant and soil materials that pose no recognised harm to the environment. Composting can also be a follow-up treatment of soil and vegetative material already inactivated through other means.
- *Desiccation* - plants without seeds can be devitalised through desiccation simply by withholding water, or they can be chopped or minced into pieces unable to grow independently under natural conditions.

Regardless of the disposal method, inactivation and decontamination must be appropriate for the organism of interest. Time and temperature criteria for the targeted organisms, autoclave test strips, and equipment maintenance and testing are but some of the tools needed for validating termination methods. Materials can be disposed with confidence once decontamination is validated.

Finally, depending on the type of work and biological material, it may be suitable to install specific containment provisions such as air curtains. Traps (e.g. light traps, sticky traps), bait and sentinel plants can help to catch unwanted insects and rodents, whilst at the same time allowing monitoring of their presence.



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### 5.3. Personal protective equipment

When choosing suitable protective clothing, two objectives should prevail:

- *Protection of workers entering the contained facility*  
When working with plants and plant pathogens, there are usually few risks for the health of the workers and of these, most are related to general occupational hazards. For instance, being regularly exposed to pollen and dust may induce allergic reactions. Similarly working between plants may lead to cuts from leaves or injuries from plant supporting systems. On this basis, some organisations require staff to wear safety glasses.
- *Avoiding that workers become a source for dissemination when leaving containment*  
No personal materials such as backpacks, coats, or bags should be allowed into containment facilities without good reason. It is recommended that lab coats are used and remain at the facility. Alternatives to dedicated clothing can be envisaged as the conditions in a glasshouse usually render wearing a lab coat very uncomfortable. Special care should also be taken to ensure that footwear do not carry GMOs from the facility. Similarly, when hands are a primary route of disseminating organisms, wearing disposable gloves is encouraged upon entry to the facility or when handling live material, and hands should be washed carefully when leaving. For entry into high-level containment glasshouses, disposable lab gowns, gloves, caps or hairnets, and/or foot coverings are usually required. This apparel must be removed before leaving the facility and decontaminated (usually by autoclaving) before washing or disposal

Whilst the main interest for containment is to avoid dissemination outside of the contained facility, restrictions may also prevail within the facility. For instance, when a person has to work the same day in different compartments, a strict order and change of personnel protective equipment may be needed to avoid carrying material from one experiment to another.

### 5.4. Practices

Irrespective of the infrastructure and equipment, overall safety and containment will be largely determined by the workers performing the activities. In order to ensure containment on a daily basis, staff must fully appreciate the specifics of the material, the containment features and the

expected behaviour. Finally, such awareness is the foundation for continuous improvement and rapid reaction in the case of any breach of containment.

Consequently, personnel instruction is a critical component of good management practices. A reference manual should be prepared containing directives covering all safety and permit considerations pertaining to the research. In addition, standard operating procedures (SOPs) can be developed describing, for example, how to use, maintain, and disinfect the facility and its equipment. SOPs should be considered 'living' documents that may be modified as new permits are received, research practices change, equipment and personnel are added, and technological innovations arise. All staff members are required to read, comprehend, and agree to adhere to the instructions provided in the manual and SOPs before entering the glasshouse. All training must be properly documented and records must be maintained.

Routine access to facilities housing confined research material is restricted, regardless of the biosafety level. Such restrictions are intended to minimise the spread of material that could be carried by people moving between rooms or facilities. An entry and exit logbook is required and for high-level containment the log may also include a description of the activity. Different levels of access authorisation may be applicable (e.g. for maintenance staff, visitors). The process for obtaining access authorisation should be clearly communicated and may involve specific training, especially when procedures must be adhered to, including those dealing with entering and leaving the facility.

As for other research settings, good hygiene practices should be standard to protect the project integrity, staff and to avoid dissemination. Eating, drinking, and smoking should be prohibited. In addition to wearing disposable gloves in some cases, hands should be washed carefully when leaving the facility. At high-level containment facilities strict personnel protective equipment and hygiene protocols are maintained. All users are required to enter only through the dressing/shower rooms and must shower when leaving the facility.

Signs must be posted at all entries, indicating that access is restricted for the research programme in progress. A description of the potential risk may be posted on the sign as long as this is not confidential information.

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The sign should state the name and telephone number of the responsible individual(s), the plants in use, and any special requirements for using the area. It may include contact information for the glasshouse manager and others to be called in the case of emergency. Information on signs should not conflict with, or compromise, security measures. Use of the universal biohazard symbol should be reserved for high-level containment areas.

For facilities designated BL2-P and higher, GM material in the form of seeds or propagules, potted plants, trays of seedlings etc. must be transferred in closed non-breakable containers. For BL3-P and BL4-P containment, some guidelines require that experimental materials are also enclosed in a secondary sealed container for transport. The exterior surface of the secondary chamber is decontaminated either chemically or in a fumigation chamber if the same plant, host, or vector is present within the effective dissemination distance of the propagules of the experimental organism.

GM material in a greenhouse room must be marked to distinguish it from non-GM organisms, such as plants serving as experimental controls or not involved with the experiment. It is recommended that GMOs have a designated boundary on the bench, using color-coded markers, for instance. In addition, individual pots, bench sections, or entire benches can be marked with stakes or signs to identify the plant and the primary genetic modification. In spite of this clear marking, any material in a contained facility is handled according to the standards dictated by the highest containment level in that facility, e.g. non-GM material will be handled as GM material. In this way, mix-up of material does not result in improper handling of waste.

Plant parts, cultures, whole plants, and seeds are routinely stored and manipulated in containment facilities. Coolers, freezers, and growth chambers equipped with locks are recommended for storage. GM seed should be stored in a locked cabinet located preferably in a glasshouse room to minimise their handling in unconfined spaces, and should be clearly identified and labelled to distinguish it from other stored seeds or materials in the cabinet. Cabinets or storage areas housing GM material must be clearly identified with signs. GM seed that is stored or handled outside the area of containment, such as in a cabinet or on a potting bench in a headerhouse corridor, should be kept in a spill-proof container. Threshers, seed counters, and related equipment used to process seed should be easy to thoroughly clean. For some operations, dedicated equipment may be

required to ensure that mixing between runs or trials does not occur.

As indicated before, special care is required to keep the facility in good hygiene conditions and to avoid that living GM material is removed via waste. This includes using well-defined disinfection and cleaning procedures and ensuring inactivation of contaminated materials and waste by validated means. For high-level containment, this is extended to include treatment of personal protective equipment before disposal or cleaning and even inactivation of effluent from hand-washing sinks and showers and similar effluents.

A pest control programme is also required to prevent dissemination throughout and outside of the facility, e.g. rodents and birds are able to transport seed, insects and other organisms can transfer pollen and pathogens to receptive plants located either within or outside the containment area, whilst viral, fungal, and bacterial organisms are not uncommon in a glasshouse setting and can cause disease when the environmental conditions favour their development on suitable host plants. Screens can exclude pollinating insects and birds, and louvers can be fitted on exhaust fans that are only open when fans are running. The perimeters of glasshouses of every containment level should be sealed to prevent rodents and other large pests from entering. Fumigation or spray application of pesticides can be used to control certain insect pests, such as whiteflies. Biological pest control measures may involve the introduction of predators, parasites, and parasitoids. Routine cleaning with hot water and detergent applied with a power washer to surfaces is a very effective method for reducing pest populations. This technique is best implemented between experimental runs.

When researchers use insect pests as part of the experimental protocol, such as in testing plants for disease or insect resistance, selective control measures are needed to eliminate unwanted pests without killing the required pest organism. When insect vectors are used to transmit GM viruses, particular care should be taken to eliminate the vector once transmission has been accomplished. A stringent pest control programme, using physical, chemical, or biological control measures, alone or in combination, should be implemented and monitored for effectiveness. Protocols should be instituted to avoid the transmission of microbial pathogens both within the glasshouse and to the outside environment.

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The growth of plants in the immediate vicinity of the facility should be restricted in order to control against the presence of sexually-compatible relatives of the experimental GM plants. This can be reasonably achieved by employing a paving or gravel barrier around the facility, in conjunction with a herbicide treatment regime.

Further control of pollen-mediated dissemination can be achieved by:

- Spatial isolation from sexually-compatible relatives in the receiving environment by ensuring that such plants are a suitable distance away from the facility,
- Temporal isolation from sexually-compatible relatives in the receiving environment by allowing the experimental plants to flower outwith the normal season.
- Physical isolation and reproductive containment by bagging flower heads of GM plants prior to anthesis using paper or glassine bags. Alternatively, experimental plants may be contained within secondary insect-or pollen-proof containers.

Similarly, seed control measures may include:

- Spatial isolation from suitable seed germination sites,
- Temporal isolation from suitable seed germination sites,
- Physical isolation and reproductive containment is sometimes possible by using a seed collection system. This will often involve bagging the flower heads and/or additional containment, such as placing the plant pots on large trays or using a proprietary collection device in order to collect as many seeds as possible,
- Sticky floor mats at the exit of the facility can be used to minimise seed dissemination on the footwear of staff.

Escaped GM organisms may be detected by placing susceptible host plants, insect traps, or spore/pollen catching devices both inside and outside of the containment area. These traps and sentinel bio-indicator plants can be used to detect unintended virus transmission, insect migration, and pollen or spore spread. Corridor light traps operated at night are useful to indicate the presence of insects that have escaped glasshouse rooms. In addition to biological systems, many of the equipment systems in a high containment facility require periodic testing to monitor efficacy. For instance, in addition to

monitoring for leaks in the glasshouse facility, it is recommended that HEPA filtration, biosafety cabinets, and sterilisation systems be checked annually. In general, glasshouses should be inspected periodically to ensure that the appropriate containment measures are rigorously applied. Inspections should be conducted on a regular schedule and whenever new types of experimental materials are brought into the facility. Inspection checklists help ensure that a glasshouse facility meets the necessary physical, biological, and managerial requirements for a given biosafety level. Re-inspections should be conducted periodically. The presence of light, heat, and water within a facility promotes gradual deterioration of equipment and structural features over time. Additionally, an inspection serves as an opportunity to review any special practices that may be required, because staff adherence to non-standard procedures has a tendency to relax over time.

The integrity of the containment facility is susceptible to equipment malfunctions, acts of nature, such as fire, flood, and storm damage, and human error. A loss of BL1-P containment due to any of these factors would likely have only minor environmental consequences. Regardless of the relatively low risk, a response is required. For BL-2P and higher containment facilities, contingency plans for handling emergency situations, including theft or vandalism should be drawn up. They must include measures to contain the breach, a personnel notification sequence, and decontamination procedures.

Vandalism is another concern. Individuals and organisations opposed to recombinant DNA research have targeted GMO research projects at both the glasshouse and field trial stage, often causing substantial damage. Determined individuals gain entry either by force, by defeating security hardware, or they may be admitted inadvertently by authorised personnel. Facility users should be advised that they share responsibility for maintaining security.

In the same context, an organisation may wish to create a response team. This group is typically composed of a high-level administrator, a public information officer, the facility manager, legal counsel, and relevant others whose job is to review physical deterrents and develop public relations strategies. Because political actions are generally designed to garner sympathy for a cause via the news media, it is important that an organisation has an opportunity to respond quickly and clearly to threats or acts of vandalism.

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## 6. CONCLUSION

Safety concerns can be elicited either by prevention, when dealing with biological agents such as pathogens and pests, or by precaution, when handling GM crops. As these drivers are fundamentally different, the risk assessment will respectively focus on known hazards or on areas of uncertainty. Irrespective, containment facilities offer an opportunity to work safely with biological material by reducing the risk of dissemination and/or exposure.

Developing a containment approach starts with understanding the specific features of the biological material and how the material will behave in the environment of the contained facility. In fact, many organisms already carry important biological containment characteristics that reduce the need for additional measures. Subsequently, physical containment measures are designed involving available infrastructure and equipment. As staff need to be able to enter the containment area to perform their activities, they need to be protected and they should also be equipped to avoid becoming a source of dissemination when leaving the area. Finally, in order to ensure maintenance of containment, procedures need to be in place for handling of the GM material and waste. At the same time, the proper functioning of containment measures needs to be controlled. Together with biological containment, physical containment provides different layers of protection, which reinforce one another.

For each containment component there are different options available, allowing the fine-tuning of containment measures to specific needs. For practical reasons, these options are usually combined in so-called biosafety levels. Whilst they provide standard procedures, containment management must take advantage of putting together combinations tailored to specific needs. As these options are sometimes very technical, it is advisable to include experienced containment glasshouse engineers in planning and design. Also, understanding how non-GM projects in plant breeding and plant pathogen are conducted safely, provides a valuable basis for design.

Risk assessments of GM crop greenhouse activities indicate that these projects can be safely conducted in the lowest level of greenhouse containment, BL1-P and BL2-P. Depending on the crop and plant development stages

handled, screenhouses can provide an adequate containment level. Activities involving GM microorganisms, pathogens and/or pests, will require a wider range -and likely also higher level- of containment measures. Nevertheless, the same approach of risk assessment based identification of appropriate measures can be applied.

Finally, authorities have followed different approaches to regulate the contained use of GM plants. Notwithstanding these differences, the objectives and basic requirements are the same. Importantly, all have included differentiated procedures to allow low risk level activities to proceed without unnecessary administrative burden.



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