HSE
Health and Safety Executive

The SACGM Compendium of guidance

Part 4: Genetic modification work that involves plants (including plant-associated genetically modified microorganisms)

HSE Books

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4.1 Genetic modification work that involves plants

Overview

1. The Genetically Modified Organisms (Contained Use) Regulations 2000 and the Environmental Protection Act 1990 (EPA) require that suitable and sufficient assessment of the risks to human health and the environment be carried out for activities involving the genetic modification of organisms. The primary role of the risk assessment is to determine the appropriate control measures that are needed to afford maximum protection to both human health and the environment. This, in turn, will determine the notification requirements for the proposed work.

2. For many activities that involve plants, or microorganisms associated with plants, the risks to human health will be outweighed by the potential for harm to the environment. Taken together, both the Contained Use Regulations and the EPA require that appropriate measures be taken to ensure that genetically modified (GM) plants or plant-associated genetically modified microorganisms (GMMs) do not cause harm to either human health or the environment. By following the advice and measures set out in this guidance, you will be doing everything that is reasonably practicable to comply with the legislation for GM activities involving plants carried out in **containment**.

3. The Genetically Modified Organisms (Deliberate Release) Regulations 2002 and the Environmental Protection Act regulate activities where a genetically modified organism (GMO) is intentionally released from someone's control into the environment. A GMO is considered 'released' if someone deliberately allows it to pass from their control into the environment without specific measures to minimise contact or harm to the general population and the environment. Under the Deliberate Release Regulations, an application for consent must be submitted to the Department for the Environment, Food and Rural Affairs (Defra). Any GMO must be authorised before it can be released into the environment or marketed within the European Union. For further information on the deliberate release regime and the application procedure, contact the GM Policy and Regulation Unit, 3/F6 Ashdown House, 123 Victoria Street, London SWE1 6DE (Tel: 08459 33 55 77, e-mail: gm@defra.gsi.gov.uk).

4. There are also controls and restrictions on the import, movement and keeping of plants, plant pests and other material (eg soil) in order to help prevent the introduction and spread of harmful organisms in the UK. The Plant Health (England) Order 2005 implements these controls in England and Wales. Similar arrangements apply in Scotland and Northern Ireland. Under the Plant Health Order, work involving genetically modified

plant pests and pathogens no longer requires notification to Defra Plant Health Division. However, if you wish to import plant pests and pathogens or receive imported plant pests and pathogens from other institutes you may still require a plant health licence. For further information on all aspects of Plant Health Licensing in England and Wales, contact Plant Health Division, Foss House, Kings Pool, 1-2 Peasholme Green, York, YO1 2PX (Tel: 01904 455174; e-mail: planthealth.info@defra.gsi.gov.uk). For further information on Plant Health Licensing in Scotland, contact The Licensing Officer, Scottish Agricultural Science Agency, 1 Roddinglaw Road, Edinburgh, EH12 9FJ (Tel. 0131 244 8957; e-mail: plant_health_licensing@sasa.gsi.gov.uk).

Scope

5. The following guidance is intended for users wishing to undertake the risk assessment of activities involving the genetic modification work involving plants in containment. The document is divided into major sections that cover certain activities:

- 4.2 to 4.3: genetic modification of microorganisms that are associated with plants; and
- 4.4 to 4.5: genetic modification of plants.

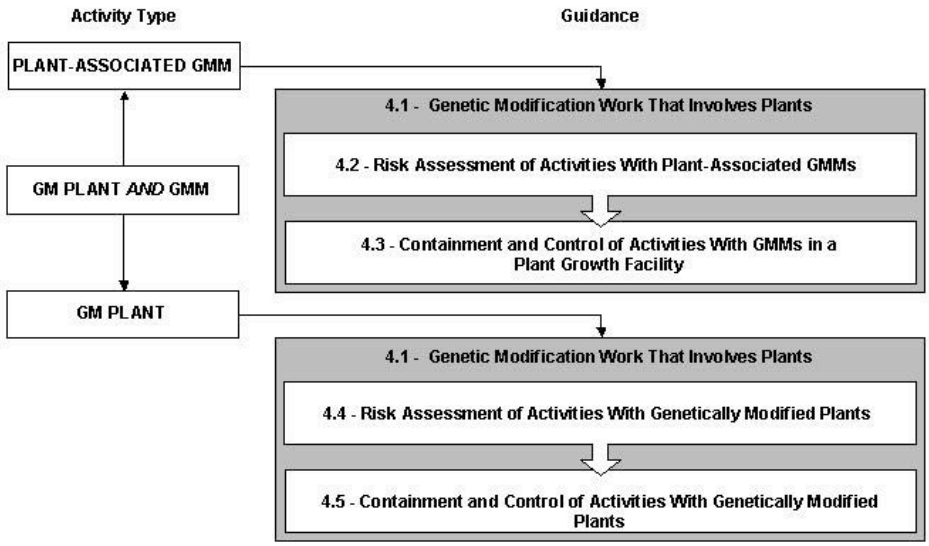

Figure 4.1.1 How to use Part 4 guidance on GM work involving plants

6. For each major activity type there is both guidance on the risk assessment of the activity, and detailed guidance relating to the assignment and implementation of containment and control measures. Users must decide which section of the guidance is most relevant for their purposes. All users should read this section (Section 4.1). For activities involving plant-associated GMMs, users should read Sections 4.2 and 4.3. For activities involving GM plants, users should read Section 4.4 and 4.5. For those wishing to undertake activities involving both GMMs and GM plants, both sections will be relevant (see Figure 4.1.1).

7. In some cases, GM plants may be exempt from majority controls under the Contained Use Regulations. For example, self-cloned plants that are unlikely to cause harm to humans are exempt, except for the requirements of regulation 17. This requires that exposure of humans and the environment, and the level of harm to humans as a result of activities involving GMOs, be reduced to the lowest level that is reasonably practicable. Self-cloning is defined as the insertion of all or part of a sequence, whether or not it has been altered by enzymatic or mechanical processes, into cells of the same or closely related species which could naturally exchange genetic material. Self-cloning could involve the use of recombinant vectors to reinsert the sequences provided that there are no genetic elements other than those designed for vector structure, replication, or maintenance. (See *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000* for further information.)

Definitions

8. For the purposes of this guidance, the term ‘plant’ is used in the broadest sense and covers higher plants, including both vegetative and reproductive organs (ie spores, seeds, pollen, bulbs, rhizomes, tubers) as well as mosses, ferns, algae and aquatic species (for example, duckweed).

9. The terms ‘microorganisms associated with plants’ and ‘GMM’ include both beneficial organisms and those considered to be plant pests. Beneficial organisms include mutualistic species such as mycorrhizal fungi, symbiotic bacteria, anti-fungal bacteria (eg fluorescent pseudomonads) and endophytic species. Plant pests are defined as organisms liable to infect plants/plant products and include pathogens of plants such as viruses, viroids, satellites, bacteria, fungi and mycoplasma, as well as plasmodiaphorids acting as intermediate vectors for viruses.

10. The term ‘vector’ refers to a microorganism used to deliver genetic information to a plant. This should not be confused with ‘intermediate vector’, which is used to describe the

carrier of a plant pathogen, such as an insect. More complex organisms that are considered plant pests, such as insects and nematodes are not covered here. Users are referred to those guidance sections within the Compendium that deal with the risk assessment and containment of GM animals.

11. Legal requirements will be stated clearly as such, with the use of the words 'regulatory requirement', 'required' or 'must'. In cases where the word 'should' is used, the guidance highlights approaches that can otherwise be used to achieve the appropriate standards. These approaches are only illustrative and users may adopt other approaches so long as the standards set by the Regulations are met.

12. The following terms do not have legal definitions and the explanations below are intended to aid understanding:

- **Laboratory:** A room in which organisms are handled or manipulated that is not a plant growth facility
- **Plant growth facility:** A structure, whether man-made or natural, permanent or impermanent that is designed and used principally for growing plants in a controlled and protected environment.
- **Required where and to extent the risk assessment shows it is required:** This indicates that the need for a particular measure is determined by the risk assessment. If the risk assessment specifies that a measure is needed for human health or environmental protection, its use is mandatory
- **Competent authority:** The body with enforcing jurisdiction over the Contained Use Regulations. In England and Wales the competent authority includes the Health and Safety Executive (HSE), the Secretary of State and the Department for Environment, Food and Rural Affairs (Defra). In Scotland the competent authority includes HSE, the Scottish Executive Environment and Rural Affairs Department (SEERAD) and the Scottish Ministers. Northern Ireland has its own separate competent authority. When seeking approval from the competent authority for particular actions, users should contact HSE in the first instance.

Risk assessments

13. Schedules 3 and 4 of the Contained Use Regulations set out the steps that should be included for the risk assessment of both GMMs and GMOs other than microorganisms (in this case, GM plants). Many of the issues raised in this guidance are exemplified using cases of GM work involving vascular flowering plants, bacteria and viruses. This merely reflects the balance of work that is undertaken in the UK and the principles of risk

assessment set out are valid and applicable to GM activities involving all plants microorganisms associated with them.

Risk assessment procedure

14. The following procedure represents a recommended model for GM risk assessments and for the assignment of containment and control measures. The procedure is reflected in the structure of the guidance. This suggested format includes the steps required for risk assessment under the Regulations, although it is not intended to be prescriptive:

    - **Risk assessment for the environment.** The identification of potential mechanisms by which the GMO might pose a hazard to plant health or the wider environment. Consideration of the potential severity, likelihood of occurrence and considerations of uncertainty. Establishment of a containment level that is sufficient to protect the environment.
    - **Risk assessment for human health.** Identification of any potential risks to human health. Consideration of the potential severity and likelihood of occurrence. Control measures needed to safeguard human health (if appropriate).
    - **Review of procedures and control measures.** Implementation of any additional control measures necessary to safeguard both the environment and human health.
    - **Determination of notification requirements.** For work with GMMs, this will require the assignment of GM Activity Class (1, 2, 3 or 4) and declaration of extra measures or derogations needed (see below).

15. It is a regulatory requirement to assess the risks and employ measures to minimise the chances of exposure or inadvertent release into the environment. It is important to identify all possible hazards to human health and the environment, especially any routes by which the GMO could be released. This will include waste disposal, equipment failure, and dissemination by humans or mechanical transmission of a plant pathogen. However, in practice these organisms should be assessed in a way that is commensurate with the actual hazards posed. There is a need is for an informed and pragmatic approach, rather than an overcomplicated assessment and unwarranted control measures.

Level of detail required

16. Much of this guidance has been prepared to aid the risk assessment of activities where uncertainty as to the nature of the intended GMO necessitates more in-depth consideration. The level of detail required will vary for from case to case and will depend upon the nature of the hazards and the degree of uncertainty. Where a potential for harm is identified, a more detailed consideration of the risks associated with the activity should

be undertaken. Equally, less detail will be required for less hazardous work, such as work with GM plants that cannot survive in the UK or activities involving disabled GM plant pathogens, particularly where there are no host species present in the receiving environment.

17. Arguments must be clear, but need not be exhaustive. The final risk assessment must contain enough background information and detail to ensure that a reviewer with a limited understanding of the precise nature of the work will not require further information to comprehend the nature of any hazards. Supplementary information can take the form of references to scientific literature and reports. All feasible potential hazards should be acknowledged and information should be based upon established scientific knowledge wherever possible. Any uncertainty should be acknowledged and dealt with appropriately; the lack of scientific evidence for a particular hazard being legitimate should not automatically be taken to mean that it does not exist.

18. All GM risk assessments should be reviewed regularly and be updated in the light of new scientific knowledge or where there has been a change in the nature of the activity (including a change in scale or any new procedures and containment measures). Documentation is important for GM work and all data should be recorded and used to supplement the risk assessment where appropriate. The risk assessment should consider the purpose of the work. For example, if the GMO is a crop species and the ultimate intention is for it to be released into the environment, then the assessment should be supplemented with relevant data obtained while it is in containment. This will aid the application for license to release that GMO under the deliberate release regime.

19. The risk assessment should include determination as to whether or not the GMO or its descendants could cause adverse effects if it escapes. In the majority of cases, containment and control measures will therefore be implemented primarily to prevent release of the GMO into the environment or to limit the impact of environmental harm. However, containment and control measures must be assigned on the basis of both environmental and human health protection. Whether or not those measures implemented for environmental protection are also sufficient to protect human health should be carefully evaluated and will be dependent upon the nature of the GMO itself. For example, GM plants used in the biomanufacture of pharmacologically active compounds may represent a greater risk to human health than will similar plants expressing genes to increase disease resistance.

Activities likely to raise safety issues

20. There are some types of work where particular caution should be exercised. These cases will generally involve work with GM plants that are able to persist in the regional environment and the handling of GM plant pathogens that are able to infect plant species growing in the UK, particularly if they are economically important crops. The following are examples of activities that warrant close scrutiny as they would represent environmental hazards in the event of an escape:

    - GM plant species likely to disturb natural ecosystems, especially derivatives of naturally-occurring species that may have a selective advantage or could disrupt the soil ecology;
    - plant-associated microorganisms with altered host interactions, including GM plant pathogens with altered tropism or host-range;
    - GM plants, or microorganisms used to infect plants, that express potentially harmful biologically active products (commonly called 'biopharming' or 'pharming').

21. GM activities involving plants will usually take place in 'standard' plant-growth facilities, such as glasshouses. This guidance is also relevant to the use of 'non-standard' growth facilities, such as the growth of GM aquatic plants in specialised containment equipment or tanks. Furthermore, consideration should be given to the use of atypical containment measures, for example GM trees grown in cages. The provisions of this guidance for the containment of some activities involving plants may not be appropriate in all cases but may be better served by guidance relating to other classes of organism. For example, growth of microalgae in fermenters might be better covered by guidance relating to large-scale growth GMMs associated with humans/animals (see Part 3, Section 3.3). Similarly, work with GM insects, nematodes and other complex plant pests will be better served covered by GM animal guidance (see Part 5).

Containment and control

22. The Contained Use Regulations require that all activities involving genetically modified organisms use a combination of physical, chemical or biological barriers to limit contact with people and the environment. With respect to work with GM or 'transgenic' plants, they only cover protection of human health. Environmental protection aspects are controlled under Part VI of the EPA, which requires that all appropriate measures are taken to avoid damage to the environment that may arise from the escape or release from human control of GM plants. By contrast, the Contained Use Regulations require the application of containment and control measures to control the risks to both humans and

the environment with respect to activities with GMMs. Therefore, taken together, the Contained Use Regulations and the EPA require that appropriate measures be taken to ensure that GM plants or plant-associated GMMs do not cause harm to either human health or the environment.

23. There are no specific measures in the legislation that are laid down for work with GM plants. Both the EPA and Contained Use Regulations require that measures be used to reduce risks to humans or the environment to either 'low' or 'effectively zero'. This guidance outlines practices and containment measures that are considered to be good practice.

24. By contrast, the Contained Use Regulations require the application of defined containment and control measures for work with GMMs, and these must be applied to any work that involves handling them (Schedule 8, Table 1a). In addition, the Regulations set out certain specific requirements for activities being undertaken in plant growth facilities (Schedule 8, Table 1b). In some cases the specified measures **modify** those for laboratory containment but in others they impose **additional** requirements. All measures are outlined in the integrated table of containment measures presented in this guidance (see Table 4.3.1).

Notification requirements

25. Regulation 9 of the Contained Use Regulations stipulates that the competent authority (HSE) be notified of the intention to begin work with any GMOs on the premises. If no previous GM work has taken place at the site, then a premises notification must be submitted to HSE. If appropriate, this can accompany an individual activity notification.

26. The risk assessment will establish the containment and control measures needed. This, in turn, will determine the notification requirements for individual activities.

- For activities involving GMMs, risk assessments are carried out under Schedule 3 and will require assignment of the work to **GM Activity Class 1, 2, 3 or 4**. Activities in Classes 2, 3 and 4 must be notified to the competent authority (HSE).
- For activities with GM plants, risk assessments for human health are carried out under Schedule 4. There is no formal requirement to assign a GM Activity Class, although this can be a useful means to determine what type of facility is appropriate (see below). **Only activities generating a GM plant with a greater potential to cause harm to human health compared to the unmodified organism require notification**. However, there remains the requirement to do an environmental risk assessment under the EPA.

27. Further information on the notification requirements can be found in Part 1 of the Compendium.

4.2 Risk assessment of activities with plant-associated GMMs

Overview

1. The following guidance concerns the risk assessment of activities involving genetically modified microorganisms that are associated with plants. This includes mutualistic or symbiotic microorganisms considered to be beneficial to plants, as well as those considered to be pathogens. Furthermore, microorganisms that have been engineered for use as biological control agents are also covered by this guidance.

2. The use of genetic modification has permitted the study of interactions between microorganisms and their host plants. This includes research into the mechanisms of pathogenesis, symbiosis and mutualism and the elucidation of plant gene functions. GM plant viruses in particular, have been exploited for both research and biotechnology applications. This is largely because transformation is only possible in a restricted number of plant species. However, plant viruses can be used to inoculate a wide range of plant species and host range can be altered. Furthermore, the use of plant virus vectors in this way overcomes the problem of position effect variegation, which occurs in GM plants that are modified by transformation.

3. For example, GM plant viruses can be used in the study of plant functional genetics by exploiting post-transcriptional gene silencing. Inoculation of a virus vector carrying a copy of the gene to be silenced triggers plant RNA-mediated defence mechanisms that counter viral threats resulting in the silencing of both the vectored gene and the cellular equivalent. This system has been dubbed virus-induced gene silencing (VIGS). GM plant viruses have also been heavily exploited for biotechnological purposes. GM plant viruses have been used to transform plants for the purposes of metabolic engineering and the expression of foreign genes, such as antigens for vaccine production and novel therapeutic products.

4. For most activities involving the genetic modification of plant-associated microorganisms, the primary considerations of the risk assessment will be given to the effects the GMM may have on plant species in the environment should it escape. This is likely to be the case for GM activities relating to the study of microbe–host interactions and plant functional genetics, as the potential ramifications for human health will be negligible. However, human health implications will require greater emphasis where activities involve genes that encode biologically active products, or products that may be toxic or allergenic. Therefore, the potential environmental impact of any GMM that can infect or interact with a plant or otherwise impact upon any environmental ecosystem (including microbial

populations) will require careful assessment and control. However, it is important not to overlook the possible effects on workers or other humans who may be exposed.

5. Each part of the risk assessment will involve the following steps:

   - hazard identification;
   - assessment of likelihood of hazards being realised, including an assessment of the relative 'fitness' of the GMM;
   - assessment of the consequences of hazards being realised;
   - determination of risk that hazards will be realised;
   - assign containment level to reduce the risks to 'effectively zero'.

6. The risk assessment process should also include a consideration of the nature of the work and a review of the procedures, with additional control measures implemented where necessary. From this, the minimum containment requirements will be evident and a GM Activity Class must be set. This will determine the notification requirements for the work.

Risk assessment for the environment

7. The objective of the risk assessment is to determine the likelihood and the possible consequences of an accidental release of a GMM from containment into the environment. In a properly maintained and managed facility with the correct containment measures in place, the likelihood of such a release will be low. However, it is important to identify all possible hazards and consider any routes by which the GMM could be released (including waste disposal, equipment failure and spread by humans).

8. The risk assessment should consider both the environment surrounding the containment facility as well as the wider environment, especially if there is a possibility that the GMM could survive and disseminate. The Contained Use Regulations require consideration of whether there may be an adverse effect from interactions of the GMM with other organisms at the premises with which it is likely to come into contact. For instance, an insect-borne pathogen and its intermediate vector may be present in adjacent laboratories. Such instances might necessitate the implementation of additional controls.

Mechanisms by which the GMM might pose a hazard to the environment

9. During the hazard identification process, the factors to consider will include:

- hazards associated with the recipient microorganism. This will be particularly relevant where the organism being modified is a plant pathogen or is not indigenous to the UK and could disrupt microbial ecological balance;
- hazards associated with the inserted gene/element. This will be particularly relevant if the insert encodes a toxic product and could have adverse effects on animals, plant and soil ecology;
- hazards arising from the alteration of existing traits. This concerns the effects of the modification and will centre upon changes to the survivability and interactions with the host plant or other environmental organisms

Hazards associated with the recipient organism

10. The characteristics of the recipient strain that will be of relevance to the final GMM include pathogenicity, virulence, infectivity, toxicity, symbiosis, ability to colonise and ability to compete with indigenous microbes. If the recipient organism is pathogenic or mutualistic, then the GMM may also exhibit the same features, albeit potentially altered by the modification.

11. Particular care must be given to the assessment of work with pathogens that infect plants that are indigenous to the UK. Clearly there may be major economic risks to consider if work is undertaken on pathogens of plants that are grown commercially. Similarly, work on pathogens that infect indigenous plants or those grown ornamentally may also pose significant hazards to the environment.

12. In the event of a release, there is potentially a fine balance between the reduced pathogenicity of an attenuated pathogen and the ability to contain an outbreak of a virulent one. Clearly, if the host organism is present in the receiving environment, then an attenuated strain should be used if possible or otherwise practicable, as this will reduce the impact of pathological effects in the event of a release. Should a virulent microorganism be used, then careful consideration should be given to the possibility that the pathogen may persist in the environment. A pathogen with increased virulence that causes severe disease (or a *hypervirulent* pathogen) might fail to persist, as the disease will be ‘self-limiting’ due to local ‘fade-out’ of the host plant population. Conversely, a less virulent strain might be more able to persist and therefore spread further. If a hypervirulent pathogen is to be constructed or used, then this should be fully justified by the risk assessment and suitable controls implemented. These activities carry with them the risk of serious environmental impact and effects upon population structure and density of the host organism, as well as impact upon the wider ecology. Such considerations need to be carefully weighed and all hazards, including the possibility of severe disease and persistence, should be fully accounted for in the risk assessment.

13. There are a number of modification strategies that can be employed to disable a plant pathogen or study mechanisms of host interactions more safely. These approaches might include:

    - deletion or mutation of genes that are essential for growth or replication;
    - deletion or mutation of genes involved in pathogenesis;
    - eliminate intermediate vector transmission by using non-transmissible isolates or altering/removing sequences required;
    - study molecular mechanisms without using whole pathogen. For instance studying self-propagating viral RNAs (*replicons*).

14. The origin and mechanism of such attenuation should be well understood and will form an important part of the risk assessment. In assessing whether a GM plant pathogen is adequately disabled, the possibility of reversion or complementation should be considered. Furthermore, it should be confirmed that the GMM is disabled, or remains so, after modification.

15. The stability of the genetic modification should also be considered, particularly where there is the possibility that an attenuated or disabled GMM might revert to a wild type or pathogenic phenotype and become an environmental hazard. The likelihood of reversion will be dependent upon the mechanism of attenuation; deletion mutants are less likely to revert than point mutations or conditional lethal mutants. Therefore, the genetic stability of the modification is linked to phenotypic stability, especially where the modification restricts the GMM's ability to survive and to spread.

16. An organism with a restricted capacity to survive will be under stress in the environment and there will be a strong selection pressure for the reversion of attenuating and disabling genetic lesions. The possibility that a GMM will be genetically unstable outside of the controlled conditions in which it was intended to exist should be taken into account and consideration given to any detrimental effects this might cause. In particular, careful consideration should be given to the use of disabled GM plant viruses in conjunction with transgenic plants engineered to complement the genes which are deleted from the viral genome (thus effectively using a 'helper plant'). Such an approach could be used to generate disabled virus vectors, providing an enhanced measure of biological containment. This approach may, however, lead to a selective pressure for recombinant viruses to reacquire the essential genes from the transgenic plant.

17. Survivability of the organism will be a key attribute. If an organism is not capable of surviving for significant periods in the environment, as may be the case for many of the

disabled organisms used in containment, then none of the other hazard areas are likely to come into play. In many cases, a disabled GMM can probably be considered safe from an environmental standpoint as they are biologically, if not physically, contained. Conversely, if an organism can survive and perhaps disseminate in the environment, then other possible hazards should be considered. This means that alterations in pathogenicity, possible adverse effects of any inserted gene products will also need to be considered.

18. When assessing whether an organism might survive in the environment, it should be remembered that this includes all types of association with living organisms, as well as the possibility of persisting in soil, water or other sites.

Hazards associated with inserted genes

19. GMMs might be a hazard to the environment by virtue of the properties inherent to the genetic insert, even if the recipient microorganism poses no specific risk. For instance, the products of the inserted sequences may have the desired effect in the intended experimental system but nevertheless kill or be detrimental for environmental plant, animal or microbial species. This is particularly relevant for modified microorganisms that could infect plants and express the inserted gene within plant tissues.

20. Careful assessment will also be required for recipient microorganisms that can remain viable outside of a plant host and secrete potentially hazardous products into soil or water. It is important to consider any potentially harmful (or beneficial) effects that a GMM could have on microorganisms in the soil environment. For instance, a soil-borne bacterium expressing and secreting anti-fungal compounds could kill mycorrhizal fungi if it escaped and became established. Similarly a plant infected with a GMM encoding a product that could disrupt mechanisms of mutualism could harm the ecology.

21. It is also important to assess the potential for an encoded product to cause adverse effects in animal populations. These considerations primarily apply to those genes encoding products with biological activities, particularly if they are novel and not normally found in plants. Examples of such genes would include those encoding industrial, pharmaceutical, immunogenic, toxic or allergenic products, such as antigens from human or animal pathogens expressed for vaccine development. Such products could have adverse effects on humans and animals in the environment. In particular, if an infectious GMM could lead to expression of a gene encoding a toxic product in a plant eaten for food by animals, then populations might be reduced.

22. It is important to consider the properties inherent to the products of a heterologous gene insert in conjunction with the expected characteristics of expression. For instance, the

gene product might be allergenic or toxic to animals. If the gene is expressed in the leaves or edible parts of an infected plant, then an adverse effect due to contact with or ingestion by animals or humans might be possible. Should the expression of that product be restricted to root tissue, then the potential risks posed to grazing animals might be reduced. However, toxic products secreted by root systems or micorrhizae might have adverse effects on soil microbial populations, symbiotic organisms and plant health. The non-coding regulatory regions and signal sequences present in the insert will affect the characteristics of expression. It is important that the effects of these are considered in addition to the biological activity of the expressed product.

23. Inserted genes might encode products with no specific activity, but nevertheless have a potentially harmful action within the GMM or due to interactions with the host. For instance, an inserted gene could encode a pathogenicity or virulence determinant. This could exacerbate a potentially harmful phenotype of a plant pest or confer pathogenicity on an organism that is otherwise harmless (see *Hazards arising from the alteration of existing traits* below). Furthermore, the insertion of an essential gene from the host plant into a GM virus vector can cause the modified virus to have harmful effects due to post-transcriptional gene silencing. If the virus is carrying an essential gene, this could have adverse effects on the growth of infected plants, overcome inherent resistance mechanisms or alter environmental tolerances.

Hazards arising from the alteration of existing traits

24. The modification may lead to adverse effects arising as the result of alteration of existing traits. This could represent an exacerbation of a pathogenic phenotype, or disruption of a mechanism that is beneficial to plant, animal or microbial populations. This may arise as the result of the product of inserted gene acting alone (see *Hazards associated with genetic inserts* above) or in combination with other microbial determinants. Alternatively it is possible that modification of normal microbial genes may also alter pathogenicity. In identifying any hazards associated with the modification to a microorganism, the following points should be considered (the list is not exhaustive):

25. ***The modification alters survivability or stability.*** A key question will be whether the modification could alter the GMM's ability to survive in the environment and this will affect whether or not other potential risk factors will come into play. Organisms will have varying degrees of survivability. However, modifications may impact upon tolerances to UV, temperature fluctuations and dehydration.

26. ***The modification alters infectivity or pathogenicity.*** Consideration should be given to modifications that might affect the pathogenic mechanisms of a GMM. For instance, the

insertion of a known pathogenicity or virulence determinant into a microorganism might increase the potential for that organism to cause harm in the event of environmental exposure. Special consideration should be given to the insertion of genes encoding products involved in pathogenesis into microorganism that are not normally harmful.

27. There are many possible mechanisms by which the inherent pathogenicity of the host organism can be affected and these may not be directly related to the harmful properties of the encoded products. Unforeseen effects may also be observed while making seemingly innocuous alterations to the genes of the organism. This is particularly relevant to complex systems such as bacteria where genes are often part of a cluster or encode a component of a regulatory network. Fungal gene regulation systems are also complex, but are poorly understood compared to bacteria. The modification or deletion of one gene may have ramifications beyond the loss or alteration of the known functions of the encoded products. The expression of other genes may be affected and biosynthetic or signalling pathways may be disrupted, resulting in altered traits.

28. ***The modification affects host plant defence mechanisms.*** The modification of genes that are involved in subverting host defence mechanisms might affect the susceptibility of plants to infection, constituting an alteration in pathogenesis. For instance, products that are secreted by bacteria can be important determinants of pathogenesis in bacteria and may suppress plant defence mechanisms.

29. ***The modification alters tissue tropism or host range.*** Modifications that could alter the types of plant tissue affected, or alter host range will require careful consideration. There are many factors that might change the natural tropism or host range of a microorganism. (The term ‘tropism’ is used here for the purposes of consistency with other parts of the Compendium. This may be taken as meaning the intentional alteration of types and location of tissues affected.) Pathogenic bacteria may also have determinants that affect host range or the ability to colonise certain sites. During the risk assessment, careful consideration should be given to the possible effects on tissues or host plants not normally affected or colonised by the recipient organism and whether the normal route of transmission of the organism has been altered. It is recognised that the consequences of changes in tropism or host range are difficult to predict. In assessing the risk of manipulations designed to modify tropism, particularly in the case of replication-competent viruses, it should be assumed that they would require higher level of containment as compared to the recipient strain until the properties of the GMM are better understood.

30. ***The modification alters transmissibility.*** A clear distinction should be drawn between the movement of a microorganism within a plant, and transmission between plants. Both

may present a hazard, although the risk assessment of the two scenarios may be very different.

31. In general, the insertion of gene sequences that are known to facilitate the migration of plant-associated microorganism within a host will potentially create a GMM that is more harmful. Careful consideration should also be give to modifying sequences that will affect the transmission between plants, for example, the DAG motif in potyvirus capsid proteins. Generally speaking, modifications that are expected to bestow additional transmissibility functions should be assumed to result in a GMM that is more hazardous.

Transfer of harmful sequences between organisms

32. There are many mechanisms by which sequences may be transferred between organisms and the factors that affect the frequency of such events and the likelihood of a harmful consequence are complex. Such issues must be carefully considered in the risk assessment. It is important to consider the potentially harmful consequences of sequences inserted into a GMM being transferred to other organisms, or that the GMM itself may acquire sequences that might result in adverse effects in the environment.

33. With the notable exception of viruses, the transfer of genetic information present on the genomes of microorganisms is much less likely than if they are present on an episomal form, such as a plasmid or cosmid. The frequencies of successful horizontal gene transfer in the environment are low, even for genes located on plasmids. However, there is a finite possibility that any gene may be transferred, even if the mechanism is just a passive one involving release of DNA from senescing cells. Therefore, the primary consideration needs to concentrate on the possible consequences, rather than on the likelihood of transfer.

34. The survival of a GMM in the environment, either independently or in association with a plant host, may affect the likelihood of nucleic acid sequence transfer to another organism. Consideration should be given to the possibility that there could be selective pressure in the environment that might contribute to the persistence of a sequence or gene and its acquisition by an organism. There are a number of mechanisms whereby sequences could be transferred or acquired. The possibility that one or more of the following mechanisms might contribute to a potentially harmful sequence being acquired by another organism should be considered:

35. ***Sequence mobilisation in bacteria.*** This is particularly pertinent to sequences that are present in a mobilisible or episomal form, such as a bacterial plasmid. Sequences present on bacterial chromosomes are less likely to be transferred.

36. ***Introduction of sequences into plant cells.*** Transformation of plants with *Agrobacterium* results in stable integration of genetic material into plant chromosomes. The genomes of some DNA plant viruses can also become inserted into plant genomic DNA.

37. ***Recombination between related viruses.*** While the phenotype of the GM virus that is under construction is the primary consideration, some thought should also be given to the possibility that harmful sequences may be transferred as the result of a recombination event. Recombination between plant viruses is common and could lead to persistence of an inserted sequence in a replication competent virus. For example, recombination is observed in geminiviruses and has been correlated with enhanced pathogenicity. Interspecies hybrids will often result in a less virulent virus but some may be more virulent than their progenitors. If a recombination event could give rise to a harmful derivative of a GM plant virus by restoring previously deleted or mutated genes, then great care should be taken to prevent cross-contamination in the laboratory or plant growth areas.

38. ***Reassortment between segmented plant viruses.*** Some viruses have segmented genomes and can achieve genetic variability in nature by 'swapping segments' with related viruses. It is important to consider that cross-contamination in the laboratory or co-infection of the GMM with a wild-type virus in the environment could result in the generation of novel strains that could be regarded as harmful.

Phenotypic and genetic stability

39. The stability of the genetic modification should also be considered, particularly where there is the possibility that a GMM attenuated or disabled for growth might revert to a wild type or pathogenic phenotype and become an environmental hazard. Therefore, the genetic stability of the modification may be linked to phenotypic stability, especially where the modification restricts the GMM's ability to survive and to spread.

40. The loss of an inserted gene from a GMM is unlikely to constitute a hazard. However, inherent genetic instability leading to incorporation of genes elsewhere in the genome of the same GMM could be hazardous. An organism with a restricted capacity to survive will be under stress in the environment and there will be a strong selection pressure for the reversion of attenuating and disabling genetic lesions. The possibility that a GMM will be genetically unstable outside of the controlled conditions in which it was intended to exist should be taken into account and consideration given to any detrimental effects this might cause.

Likelihood that the GMM will be a risk to the environment

41. The initial stages in the risk assessment process thus far involve identifying those features of the GMM that have the potential to cause harm and the mechanisms by which these hazards could be realised. While it may be possible to draw up theoretical scenarios whereby the GMO may be hazardous to the environment, the chances of them being realised should be evaluated and understood.

42. It is therefore important to consider the likelihood that the identified hazards will be manifested. Factors that come into play are: (i) judgements as to the overall fitness of the GMM; (ii) the probability that rare events may occur (eg the likelihood of gene transfer); and (iii) the severity of the possible consequences.

43. Estimating the likelihood of a harmful consequence being realised will be difficult where there is no firm data on which to base a judgement. In general, the weight given to information used in these considerations should reflect the quality of the supporting data. Where the likelihood of harm is poorly understood, a cautious approach should be adopted until evidence to the contrary has been obtained.

Assessment of likelihood

44. A key factor in whether or not the hazard will be realised is the environment into which the GMM would be released. It is therefore important to consider the nature of the GMM in relation to the receiving environment. There may be characteristics of the receiving environment that will contribute to the likelihood of the hazard being manifested, for example the presence of a suitable host species or soil conditions. For the purposes of using the risk determination matrix, likelihood can be expressed as ‘high’, ‘medium’, ‘low’ or ‘negligible’.

45. Even if the GMM could conceivably survive, become established and disseminate in the environment, it may be that the environment itself would not be able to support it. For example, GMMs derived from pathogens of plants that are not present in the UK would have limited capacity to become disseminated, even if it could survive for extended periods. Similarly, the transmission of some pathogens may require an intermediate vector that might not be present in the UK. The possibility of unknown hosts or intermediate vectors should be accounted for, as should the longer-term possibility that such hosts and vectors will become native to the UK, for example, as a result of climate change. However, in general, the risk that such GMMs could be a hazard to the environment will be negligible.

Consideration of the ability of the GMM to become established

46. An assessment should be made as to the ability of the GMM to become established, how efficient it will be and its ability to spread within a host, population or ecosystem. This represents an evaluation of the 'fitness' of a GMM and should be based upon available scientific knowledge. Any uncertainty should be acknowledged and the precautionary principle followed.

47. The concept of fitness is difficult to define but will clearly be important in assessing the potential for a GMM to cause harm if there were to be a breach of containment. For instance, over-expression of a toxin in a bacteria or fungus may make the GMM more hazardous than the recipient strain, but the over-expression of that toxin might be deleterious to the metabolism of the organism.

48. An example relating to fitness has been demonstrated with a number of GMM systems, as there is a tendency for inserted sequences to be deleted. The loss of a gene that confers environmental tolerances would therefore reduce the potential for spread and render the virus less fit. However, extra gene carriage should not automatically be presumed to reduce GMM fitness.

Consideration of the probability that rare events will occur

49. It is often possible to assign a frequency to a given event, for example, mutation, recombination or sequence mobilisation rates. Often, this can take the form of a precise numerical frequency obtained in-house or through published data.

50. In many cases, precise evaluation will not be possible or properly supported. An approximate, semi-quantitative or descriptive assessment of the frequency, based upon experience with similar GMMs or techniques, could be used in these cases. For example, the likelihood of an attenuated or disabled GMM reverting to wild-type status can be assessed on the basis of the number of discrete events that would need to take place, ie the more events needed, the less likely it is that reversion will occur.

51. However, it should not be assumed that failure to observe an event is evidence that it does not occur. As part of such considerations it should be recognised that microorganisms often have extremely short generation times and adapt to specific environments and selective pressures rapidly.

52. Mutant genomes are continually being generated and the effects of selection pressures should be assessed. For example, although variants will be often be maintained at low

frequencies by negative selection, in a situation where a microorganism can replicate in an environment that differs from that in which it is normally found, the probability of one of the genetic variants becoming dominant will be increased. When undertaking risk assessments of GMMs it is important to have some awareness of this genetic variability. Even if the GMM that is initially constructed is not well adapted to growth in a particular environment or host, there is a possibility that it will adapt as new variants arise. Therefore, it is necessary to proceed with caution and use defective recipient strains wherever possible. This will virtually eliminate problems arising from genetic variability.

53. When estimating the probability and frequency of events, consideration should also be given to the number of organisms that might be involved in the incident. This will depend on the nature of the experiment. However the probability that a hazard will be realised will often depend on the number of GMMs that are being handled and, consequently, the number that could escape.

Assessment of the possible consequences

54. After the likelihood of all hazards is assessed, the consequence of each hazard should be estimated. Again, the consequence will depend to a very large extent on the potential receiving environment. In particular, the presence of compatible host plants or species with which the GMM may be able to compete will be important considerations.

55. Evaluation of the magnitude of potential consequence is difficult since there is inevitably a degree of judgement involved, although a qualitative appraisal of the impact on other species or ecosystems should be possible. For the purposes of using the risk determination matrix Table 4.2.1, consequences could be described as being ‘severe’, ‘modest’, ‘minor’, or ‘negligible’. The following descriptions may help:

- **Severe consequence:** a major change in the numbers of one or more species leading to negative effects on the functioning of the ecosystem and/or other connected ecosystems (for example, significantly altering the turnover of biomass, or supply of nutrients to crops). It is unlikely that the changes would be easily reversible.
- **Negligible consequence:** no measurable change in any population eg plant, animal or microbial, in the environment or in any ecosystem function. (This does not preclude some fluctuation in indigenous populations as long as this is within the range of that which could be expected naturally.)

56. It should be borne in mind that even if the consequences of a hazard being realised are deemed ‘severe’, if the probability of the hazard being manifested at all was ‘negligible’ then there is ‘effectively zero’ risk of harm. Likewise if the consequence of a hazard were

'negligible' or 'minor', then even if the probability of its manifestation were 'high' the risk of harm would still be 'low' (see Table 4.2.1).

57. However, a cautious approach to risk determination is advised. In situations where the probability of the hazard being manifested was 'negligible', should there be a 'severe' consequence to the identified hazard, then more stringent containment than would otherwise be appropriate for an 'effectively zero' risk of harm might be prudent. A balanced view of the risks is therefore required.

Determination of risk

58. The following determination matrix can be used to estimate the level of risk. This matrix is provided as a tool and is not intended to be a definitive measure of risk.

		Likelihood of hazard			
		High	**Medium**	**Low**	**Negligible**
Consequence of hazard	**Severe**	High	High	Medium	Effectively zero
	Modest	High	Medium	Medium/low	Effectively zero
	Minor	Medium/low	Low	Low	Effectively zero
	Negligible	Effectively zero	Effectively zero	Effectively zero	Effectively zero

Table 4.2.1 Risk determination matrix

59. It may be necessary to evaluate whether any specific control measures are required to adequately protect the environment. Containment measures should be applied until the risk of harm is 'effectively zero'. Further guidance on containment measures to protect both the environment and human health can be found below.

Containment level needed to sufficiently protect against harm to the environment

60. It is recommended that the minimum containment level (Containment Level 1, 2, 3 or 4) that is necessary to protect the environment be set. At this stage, it is only an estimate of the containment measures that will be required solely for the purpose of preventing release of the GMM or to minimise the likelihood that it will become a threat to the environment. Factors that may be relevant to this include:

- containment measures required by any plant health license needed for work on the **recipient** microorganism where it is an unmodified plant pathogen;
- any identified hazards arising as a consequence of the genetic modification, the severity of any harmful consequences and the likelihood that they might occur (determination of the risk of harm, see above).

61. If there are no prescribed containment measures for the recipient organism, then a judgement should be made about whether the GMM will be a risk to the environment. If all risks are deemed to be 'low' or 'effectively zero' then no specific measures will be required. However, if any risk exceeds this level then control measures should be implemented such that the risk of harm to the environment is reduced to 'low' or 'effectively zero'.

62. Users should judge which measures listed in the appropriate tables of containment measures in Schedule 8 to the Contained Use Regulations (which are reproduced in Section 4.3) are appropriate for containment of the GMM. The containment level can be set accordingly to safeguard the environment. It is recognised that there is a degree of judgement required in setting 'risk values' and containment measures. Specific advice on risk assessment and containment is available from HSE.

Risk assessment for human health

63. It is recognised that for many activities with GM plant-associated microorganisms, the risk to humans will automatically be low or effectively zero. The objective is to identify any plausible hazards to human health and then to assess the likelihood and potential severity of the consequences, should the hazards be realised. Where a hazard is identified, this will most likely be associated with modifications that result in production of a toxin or allergen. Biomanufacture may involve the transformation or transduction of a plant with a GMM, resulting in the production of pharmacologically or immunologically active substances.

Mechanisms by which the GMO could be a risk to human health

64. As for the environmental risk assessment, the hazard identification process must include considerations of potentially harmful or adverse effects upon human health that would be mediated by the recipient organism, the products of any inserted genes or the predicted properties of the final GMM. However, assessments should concentrate on hazards arising from modification, rather than those associated with the recipient organism.

65. The majority of human health hazards will most likely arise where toxic products are secreted by a GMM. Alternatively, hazards may arise as a result of modifications that alter properties of an infected plant. Using a GMM as a vector in plants that express biologically active compounds might them more toxic or allergenic.

66. Where a potential for harm to humans is identified, consideration should be given to whether direct contact with GMM-contaminated material, or with transduced plant materials (eg leaves, sap or pollen) might represent a hazard. Consideration may also need to be given to the potential for the products to be expressed in different plant tissues, the consequent routes of exposure and the possibility that these may be altered.

67. Consideration should also be given to the possibility that microbial or plant post-translational processing may differ from mammalian cells. Therefore, potentially toxic or allergenic human or animal products expressed in microbial or plant systems might be processed differently and there may be unexpected effects due to presentation of novel confirmations.

Likelihood that the GMM will be a risk to human health

68. For each identified hazard an estimation of the likelihood of it being manifested and the seriousness of the consequence should be made in a similar way to the assessment of environmental risks outlined above. The GMM may have characteristics that might lead to a potential health hazard, but the chances of them being realised should be evaluated and understood. The risk determination matrix can be used as a tool to evaluate the magnitude of the hazards. This will require an estimation of both the likelihood and consequences of exposure. This matrix is not intended to be a definitive measure of risk and the specifics of each case should be carefully considered.

69. Once again, estimating the likelihood of a harmful consequence being realised will be difficult where there is no firm data on which to base a judgement and the weight given to information should reflect the quality of the supporting data. Where the likelihood of harm is poorly understood, a precautionary approach should be adopted until evidence to the contrary has been obtained. For the purposes of using the risk determination matrix, likelihood can be expressed as ‘high’, ‘medium’, ‘low’ or ‘negligible’.

70. Similarly, evaluation of the magnitude of potential consequence may be difficult as it is inevitable that this will involve a degree of judgement. However, a qualitative appraisal of the impact on humans should be possible. For the purposes of using the risk

determination matrix, consequences could be described as being ‘severe’, ‘modest’, ‘minor’, or ‘negligible’.

Containment level needed to sufficiently protect human health

71. It is recommended that the minimum containment level (Containment Level 1, 2, 3 or 4) that is necessary to protect human health be set. At this stage, it is only an estimate of the containment measures that will be required solely for the purpose of safeguarding the well-being of those who may come into contact with the GMM.

72. The measures implemented for environmental protection may be adequate to protect human health. In many cases, the principles of good occupational safety and hygiene and good microbiological practice will also be sufficient for this purpose. These principles are detailed in Part 3, Section 3.1. However, it may be necessary to evaluate whether any specific control measures are required to protect human health. If necessary, containment measures should be applied until the risk of harm is ‘effectively zero’. It is a requirement of the Contained Use Regulations that all measures deemed by the risk assessment as necessary for the protection of human health be implemented.

73. Users should judge which measures listed in the appropriate tables of containment measures in Schedule 8 to the Contained Use Regulations (which are reproduced in Section 4.3) are required to minimise harm to workers exposed to the GMM. The containment level can be set accordingly.

Review of procedures and control measures

74. The requirements of the final containment level must be sufficient to control all the potential harmful properties of the GMM and offer sufficient protection for both the environment and human health. All risks must be reduced to ‘low’ or ‘effectively zero’. The containment and control measures identified so far for environmental and human health protection only broadly define those needed as a function of the properties of the GMM itself.

75. The nature of the activity will also affect the level of risk. Therefore, it is important to take into account the nature of the work or any non-standard operations that might increase the likelihood of release or risk of exposure. For example, large-scale growth or harvest of a GMM will often mean that large amounts of the organism will be handled, which may result in increased likelihood of release and/or exposure.

76. If any such operations or activities are likely to generate risks that are not accounted for in the minimum containment measures already applied in reaction to the risk assessments for the environment and human health, then additional control measures should be applied. Equally, it may be that as a result of the nature of the activity, the nature of a risk that is inherent to the GMM itself is diminished. For example, if GMMs are cultured in a sealed system, then exposure to workers might be much less likely. In these cases, certain control measures might not be required.

77. The person responsible for the work should be satisfied that the local rules covering the use of laboratories or plant growth facilities are adequate to minimise or prevent viable GMMs being released from the containment facility. Moreover there should be a programme of internal inspections and/or active monitoring to ensure that the local rules are satisfactorily implemented. All workers should be trained in good laboratory or glasshouse techniques before commencing work and should be fully aware of the potential hazards inherent to the activity. Access to the containment facilities should be limited, where appropriate, to authorised personnel and designated workers.

78. The maintenance schedule for protective apparatus such as safety cabinets and ventilation systems should be strictly adhered to. It is also important that the fabric of the facility and control measures (eg mesh guards on drains and vents) are regularly checked for possible breaches in containment. One of the major release routes will be via contaminated waste and it is therefore important that GMMs that pose an environmental hazard are adequately inactivated and appropriately disposed of. Further guidance on containment and control strategies and waste inactivation can be found in Part 3.

Assignment of GM Activity Class

79. A GM Activity Class must be assigned in relation to the control measures needed to protect both the environment and human health (ie Class 1, 2, 3 or 4) for work with GMMs. The measures that are indicated as necessary by the risk assessment must be applied.

80. The importance of the final activity classification is twofold:

- It determines the minimum containment and control measures that must be applied. For Class 1 activities, Containment Level 1 measures must be applied as a minimum. For Class 2 activities, Containment Level 2 and so on. The only exception to this is when the user has the agreement of the competent authority to not apply the full corresponding containment level.

- It determines the notification requirements for the activity (see Part 1 and Figure 1.0.2).

81. The risk assessment must be used to determine the appropriate control measures that are needed to afford maximum protection to both human health and the environment. The Contained Use Regulations state that 'a person who undertakes an activity involving genetic modification of micro-organisms shall apply the containment measures set out in the applicable Table in Schedule 8, where and to the extent required in the column of the appropriate containment level'.

82. For activities with plants that involve handling GMMs, in addition to the measures set out in Schedule 8 Table 1b (*Containment measures for activities involving genetic modification of microorganisms in plant growth facilities*) the relevant containment measures from Schedule 8 Table 1a (*Containment measures for activities involving genetic modification of microorganisms in laboratories*) must also be applied. Therefore, users may wish to read *Containment and control measures for laboratory activities involving genetically modified microorganisms* in conjunction with this guidance (Part 3, Section 3.2). However, the table represented in this guidance has been integrated such that all relevant measures for activities with GMMs associated with plants are shown.

83. To decide on the final classification, users should therefore compare the measures warranted by the risk assessment with the integrated table of containment measures (Table 4.3.1). Where the required containment measures correspond to those from a single level of containment this process will be simple: a GM activity requiring Containment Level 2 will be GM Activity Class 2. There will be cases, however, where the required containment measures are a mixture from two levels, for instance, Containment Level 2 with the addition of one or two measures from Level 3. Where there is such a mixture of containment measures, the GM Activity Class will correspond to the higher level of containment indicated (which, in this case, is Class 3) and must be notified accordingly. However, derogation may be sought from HSE at notification to exclude those measures required for the higher containment level that are shown to be superfluous by the risk assessment. Further explanation of the classification system can be found in the *A guide to the Genetically Modified Organisms (Contained Use) Regulations 2000*.

84. Some control measures deemed necessary by the risk assessment may not be listed in the Schedule 8 containment level tables. The GM Activity Class is determined solely by those measures actually listed. The risk assessment must always take precedence and **all** measures identified as necessary must be applied (there is a general requirement for the exposure of humans and the environment to GMMs to be as low as reasonably

practicable and the principles of good microbiological practice and of good occupational safety and hygiene must also be applied).

Risk assessment

▼

Containment measures needed to control the risk

▼

GM Activity Class

▼

Containment measures that must be applied

85. In the majority of cases, the containment measures necessary to control the risk, the GM activity class and the minimum containment level to be applied will be the same:

Containment measures needed to control risk indicate:

Containment Level 2

▼

GM Activity Class is *Class 2*

▼

Containment Level 2 applied

86. The containment measures indicated by the risk assessment may consist of a mixture of measures from two different levels. In these cases, the higher level of containment will determine the GM Activity Class and must be applied. A request can be made to the competent authority at the time of notification for permission to use the mixture of two levels identified, but unless and until you have the agreement of the competent authority, you may not use a level of containment lower than that corresponding to the GM Activity Class.

Containment measures needed to control risk indicate:

Containment Level 2 plus additional measures from *Containment Level 3*

▼

GM Activity Class is *Class 3*

▼

Containment Level 3 applied

(with derogations for those measures which are not required, under agreement with the competent authority)

Further aspects of activity classification

87. Class 1 activities are described in the Contained Use Regulations as being of 'no or negligible risk'. It is unlikely that any non-disabled plant pathogen could be deemed to be of 'no or negligible risk' (except where the host species is absent from the receiving environment) and such work will always be GM Activity Class 2 or higher. Since work with pathogens will almost invariably require at least some of the measures required at Containment Level 2 (eg an autoclave in the building; restriction of access) it would not normally be possible to assign the activity to Class 1.

88. The containment measures needed for work with pathogens of plants species not indigenous or present in the UK should be assessed on a case-by-case basis. However, if a GMM that is pathogenic and transmissible to plants that are present in the receiving environment is assigned to GM Activity Class 1, then it is probable that the risk assessment is inadequate and the activity may require notification to HSE as Class 2.

89. Remember that classification into a GM Activity Class does not necessarily mean that you will always have to apply all the measures from the associated containment level. If it is adequately justified by the risk assessment, derogation may be sought from HSE to exclude unwarranted measures.

90. Further guidance on the application and implementation of control measures at the various containment levels can be found below.

4.3 Containment and control of activities with GMMs in a plant growth facility

Containment measures		Containment Level		
		1	2	3
Building				
M1	Permanent structure	required where and to extent the risk assessment shows it is required	required	required
M2	Laboratory suite: isolation	not required	not required	required
M3	Laboratory: sealable for fumigation	not required	not required	required
Equipment				
M4	Surfaces impervious to water and resistant to acids, alkalis, solvents, disinfectants, decontamination agents and easy to clean	required for bench	required for bench	required for bench and floor
M5	Entry via an airlock or a separate room with two interlocking doors	not required	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required
M6	Negative pressure relative to the pressure of the immediate surroundings	not required	required where and to extent the risk assessment shows it is required	required
M7	Extract and input air from the laboratory should be HEPA filtered	not required	not required	HEPA filters required for extract air
M8	Microbiological safety cabinet/enclosure	not required	required where and to extent the risk assessment shows it is required	required, and all procedures with infective materials required to be contained within a cabinet/enclosure
M9	Autoclave	required on site	required in the building	required in the laboratory suite
M10	Control of contaminated run-off water	required where and to extent the risk assessment shows it is required	required so as to minimise run-off	required so as to prevent run-off
System of work				
M11	Access restricted to authorised personnel only	not required	required	required
M12	Specific measures to control aerosol dissemination	not required	required so as to minimise	required so as to prevent
M13	Shower	not required	not required	required where and to extent the risk assessment shows it is required
M14	Protective clothing	suitable protective clothing required	suitable protective clothing required	suitable protective clothing required; footwear required where and to extent the risk assessment shows it is required
M15	Gloves	not required	required where and to extent the risk assessment shows it is required	required

Containment measures		Containment Level		
		1	2	3
M16	Effective control of disease vectors such as insects, rodents, arthropods which could disseminate the GMM	required	required	required
M17	Effective control of pollen, seeds and other plant material which could disseminate the GMM	required where and to extent the risk assessment shows	required so as to minimise dissemination	required so as to prevent dissemination
M18	Procedures for transfer of living material between the plant growth facilities, protective structure and laboratory shall control dissemination of GMMs	required so as to minimise dissemination	required so as to minimise dissemination	required so as to prevent dissemination
M19	Specified disinfection procedures in place	required where and to extent the risk assessment shows it is required	required	required
Waste				
M20	Inactivation of GMMs in effluent from handwashing sinks and showers and similar effluents	not required	not required	required where and to extent the risk assessment shows it is required
M21	Inactivation of GMMs in contaminated material and waste	required by validated means	required by validated means	required by validated means, with waste inactivated in the laboratory suite
Other measures				
M22	Laboratory to contain its own equipment	not required	not required	required, so far as is reasonably practicable
M23	An observation window or alternative is to be present so that occupants can be seen	required where and to extent the risk assessment shows it is required	required where and to extent the risk assessment shows it is required	required
M24	Safe storage of GMMs	required where and to extent the risk assessment shows it is required	required	required
M25	Written records of staff training	not required	required where and to extent the risk assessment shows it is required	required

Table 4.3.1 Containment measures

Note: Containment Level 4 is not represented in the table or the associated guidance. No such facility currently exists in the UK and it is not envisaged that any work involving GMMs in association with plants will warrant the use of Containment Level 4. If such work is proposed, or the construction of such a facility is planned then it is strongly advised to discuss the details of containment requirements, management control, design of the facility etc in advance with the competent authority.

Containment Level 1 for GMMs in a plant growth facility

1. Containment Level 1 must be applied for Class 1 activities involving GMMs. All the required measures, in addition to the principles of good microbiological practice and good occupational safety and hygiene (GOSH) (see Part 3, Section 3.1), must be applied unless agreement has been obtained from the competent authority.

Building

M1. It is permissible to use a non-permanent structure. Facilities such as basic polytunnels may be appropriate, although the structure should be of suitable design and construction and be appropriately maintained so as to withstand normal climactic conditions over the period of the activity. Where the facility is a glasshouse it shall have a continuous waterproof covering. Permanent structures must have self-closing, lockable outer doors and be located on a site designed to prevent the entry of surface run-off water. It is recognised that most facilities of this type have doors that are not self-closing. Provided doors are not left open when the facility is not in use, users may consider this requirement to be met.

M2. There is no regulatory requirement for a Containment Level 1 facility to be physically separated from other areas of the building. There should be adequate space provided and the working area should be a safe, comfortable environment that takes full account of work practices and equipment present.

M3. A Containment Level 1 facility does not need to be sealable for the purposes of fumigation. However, there may be a requirement for specific disinfection procedures to be in place (see M20).

Equipment

M4. There is a regulatory requirement for the bench surfaces to be easily cleaned, be impervious to water and resistant to acids, alkalis, solvents, disinfectants and other decontamination agents that may be in use. It is recognised that the benching commonly used within plant growth facilities may not be impervious to water. Such benching is frequently made of mesh in order to permit the free drainage of water. Where such benching is used, run-off water should be controlled by alternative means, eg using saucers and trays (see M10). Although not required to have a permanent floor, the facility should be easy to clean in order to maintain good hygiene levels. Plastic sheeting, or other flooring material should be used in conjunction with clearly defined walkways to reduce the spread of GM material within the facility.

M5. Not required

M6. Not required

M7. Not required

M8. There is no regulatory requirement for the use of a microbiological safety cabinet or other similar equipment and all work can take place on the open bench. It is acknowledged that containment equipment such as a microbiological safety cabinet might be in use to prevent contamination of the GM work or products being handled.

M9. An autoclave is required to be available on the site, but not necessarily in the same building. However, it is recognised that, in some cases, autoclaving will not be appropriate and alternative waste inactivation procedures should be used. In these cases, derogation must be requested from the competent authority detailing the alternative waste management procedures in place (see M21).

M10. Where the risk assessment identifies that a GMM could be disseminated via the drainage system, control measures must be used to control run-off water. No plants should be planted directly into the ground. All higher plants should be grown in pots, trays or similar containers. All lower plants should be grown in physical containers such as flasks, tanks or fermenters. This could be supplemented with appropriate filters/mesh covers to limit the amount of soil, plant material and water entering the drains. The facility is not required to have a dedicated drainage system and therefore soakaways may be sufficient.

System of work

M11. There is no requirement for access to Containment Level 1 laboratories to be restricted. However, those permitted to work in the laboratory should be competent, trained and properly informed (see M25).

M12. Procedures should be carried out in such a way as to keep aerosol production to a minimum but there is no requirement for specific measures to control aerosol dissemination. Care should be taken to ensure that contact of the GMMs with people and the environment is minimised.

M13. There is no requirement for a showering facility to be present and workers are not required to shower when entering or leaving the facility. However, good hygiene should

40. The sequence of the inserted gene could be manipulated to reduce the likelihood of a transencapsidation event. For instance, a mutated coat protein construct that produces a protein incapable of being assembled into virus particles could be used. For example, mutation of the TMV coat protein at residue 28 has been shown to prevent virus particle assembly, while maintaining its inherent ability to protect tobacco against wild-type TMV infection. Similarly, in potyviruses the amino acid motif DAG must be present near the N-terminus of the coat protein to allow aphid transmission; mutations at this site render the virus non-transmissible by vectors.

Assessment of consequence

41. After the likelihood of all hazards is assessed, the consequence of each hazard should be estimated. Again, the consequence will depend to a very large extent on the potential receiving environment. In particular, the presence of sexually compatible plants or species with which the GMO may be able to compete will be important considerations.

42. Evaluation of the magnitude of potential consequence is difficult since there is inevitably a degree of judgement involved, although a qualitative appraisal of the impact on other species or ecosystems should be possible. For the purposes of using the risk determination matrix in Table 4.2.1, consequences could be described as being 'severe', 'modest', 'minor', or 'negligible'. The following descriptions may help:

- **Severe consequence:** a major change in the numbers of one or more species leading to negative effects on the functioning of the ecosystem and/or other connected ecosystems (for example, significantly altering the turnover of biomass, or supply of nutrients to crops). It is unlikely that the changes would be easily reversible.
- **Negligible consequence:** no measurable change in any population, eg plant, animal or microbial, in the environment or in any ecosystem function. (This does not preclude some fluctuation in indigenous populations as long as this is within the range of that which could be expected naturally.)

43. It should be borne in mind that even if the consequences of a hazard being realised are deemed 'severe', if the probability of the hazard being manifested at all was 'negligible' then there is 'effectively zero' risk of harm. Likewise if the consequence of a hazard were 'negligible' or 'minor', then even if the probability of its manifestation were 'high' the risk of harm would still be 'low' (see Table 4.2.1).

44. However, a precautionary approach to risk determination is advised. In situations where the probability of the hazard being manifested was 'negligible', should there be a 'severe' consequence to the identified hazard, then more stringent containment than would

otherwise be appropriate for an 'effectively zero' risk of harm might be prudent. A balanced view of the risks is therefore required.

Determination of risk

45. The risk determination matrix can be used to estimate the level of risk (see Table 4.2.1). This matrix is provided as a tool and is not intended to be a definitive measure of risk. It may be necessary to evaluate whether any specific control measures are required to adequately protect the environment. Containment measures should be applied until the risk of harm is 'effectively zero'. Further guidance on containment measures to protect both the environment and human health can be found below.

Containment level needed to sufficiently protect against harm to the environment

46. It is recommended that the minimum containment measures needed that are necessary to protect the environment be set at this stage. The containment measures that will be required will be solely for the purpose of preventing release of the GMO, or to minimise the likelihood that it will become a threat to the environment. Factors that may be relevant to this include:

    - containment measures required by any plant health license needed for work on the **recipient** organism;
    - any identified hazards arising as a consequence of the genetic modification, the severity of any harmful consequences and the likelihood that they might occur (determination of the risk of harm, see above).

47. If there are no prescribed containment measures for the recipient organism, then a judgement should be made about whether the GMO will be a risk to the environment. If all risks are deemed to be 'low' or 'effectively zero' then no specific measures will be required. However, if any risk exceeds this level then control measures should be implemented such that the risk of harm to the environment is reduced to 'low' or 'effectively zero'.

48. There is no regulatory requirement to set a formal containment level (ie Containment Level 1, 2, 3 or 4) for work with GM plants. A number of containment measures and procedures that can be used to reach the required standards can be found in Section 4.5. However, many users find it helpful to set a containment level that is appropriate for the facility in which the work will be carried out. *A guide to assigning containment levels for activities with GM plants* can be found below. Users could judge whether the measures in the integrated table (Table 4.3.1) in Section 4.3 of containment measures are also

appropriate for the GM plant. This might be particularly appropriate to situations where the GM plant will be handled in the same facility in which work with a GM microorganism is taking place. If using this approach, however, it is important to remember that the Environmental Protection Act requires the containment measures used to be sufficient to safeguard the environment. Therefore, additional measures may be required beyond those in the table.

Risk assessment for human health

49. There is a requirement under the Contained Use Regulations to consider risks to human health posed by the GM activity. The objective is to identify all plausible hazards to human health and then to assess the likelihood and potential severity of the consequences, should the hazards be realised. It is recognised that for many activities with GM plants, the risk to humans will automatically be low or effectively zero. However, hazards to humans might arise due to modifications that affect allergenicity or toxicity of a plant.

50. Guidance on containment and control strategies that are relevant to plant growth facilities can be found in Section 4.5.

Mechanisms by which the GMO could be a risk to human health

51. As for the environmental risk assessment, the hazard identification process must include considerations of potentially harmful or adverse effects upon the environment that would be mediated by the recipient organism, the products of any inserted genes or the predicted properties of the final GMO. However, assessments should concentrate on hazards arising from modification, rather than those of the parent plant.

52. The majority of human health hazards will most likely arise due to modifications that alter allergenic or toxic properties. For instance, the expression of genes encoding biologically active compounds might result in plants that are more toxic or allergenic. For example, plants that are used in the biomanufacture of pharmaceuticals and other bioactive products, eg Aprotinin, Interferon, MAbs, Alpha-galactosidase A and Lysosomal Acid Lipase, may represent a greater risk of harm to human health than plants expressing marker genes. Alternatively, the exacerbation of the inherent properties of an already toxic plant might represent a mechanism whereby the hazards to human health are increased. For instance, a modification that results in elevated levels of atropine expression in *Atropa belladonna* would arguably represent a GMO that is of a greater risk to human health than the wild-type plant.

53. Where a potential for harm to humans is identified, consideration should be given to whether direct contact with plant parts (eg leaves, sap or pollen) might be a hazard, or whether the plant could be incidentally or inadvertently ingested. Consideration may also need to be given to the potential for the products to be expressed in different plant tissues, the consequent routes of exposure and the possibility that these may be altered.

54. Consideration should also be given to the possibility that plant post-translational processing may differ from that of mammalian cells. Therefore, potentially toxic or allergenic human or animal products expressed in plant systems might be processed differently and there may be unexpected effects due to presentation of novel confirmations.

Likelihood that the GMO will be a risk to human health

55. For each identified hazard an estimation of the likelihood of it being manifested and the seriousness of the consequence should be made in a similar way to the assessment of environmental risks outlined above. The GMO may have characteristics that make it a potential health hazard, but the chances of them being realised should be evaluated and understood. The risk determination matrix (Table 4.2.1) can be used as a tool to evaluate the magnitude of the hazards. This will require an estimation of both the likelihood and consequences of exposure. This matrix is not intended to be a definitive measure of risk and the specifics each case should be carefully considered.

56. Once again, estimating the likelihood of a harmful consequence being realised will be difficult where there is no firm data on which to base a judgement and the weight given to information should reflect the quality of the supporting data. Where the likelihood of harm is poorly understood, a precautionary approach should be adopted until evidence to the contrary has been obtained. For the purposes of using the risk determination matrix, likelihood can be expressed as ‘high’, ‘medium’, ‘low’ or ‘negligible’.

57. Similarly, evaluation of the magnitude of potential consequence may be difficult as it is inevitable that this will involve a degree of judgement. However, a qualitative appraisal of the impact on humans should be possible. For the purposes of using the risk determination matrix, consequences could be described as being ‘severe’, ‘modest’, ‘minor’, or ‘negligible’.

Control measures needed to sufficiently protect human health

58. It may be necessary to evaluate whether any specific control measures are required to adequately protect human health. If necessary, containment measures should be applied

until the risk of harm is ‘effectively zero’. It is a requirement of the Contained Use Regulations that all measures deemed by the risk assessment as necessary for the protection of human health be implemented.

59. In many cases, the relevant principles of good occupational safety and hygiene will be sufficient to protect human health. These principles are detailed in Part 3, Section 3.1. Furthermore, some of the measures implemented for environmental protection may be adequate to minimise or prevent exposure. However, only risks to human health will have a bearing on the notification requirements for the work (see below).

Review of procedures and control measures

60. The requirements of the final containment level must be sufficient to control all the potential harmful properties of the GMO and offer sufficient protection for both the environment and human health. The containment and control measures identified so far for environmental and human health protection only broadly define those needed as a function of the properties of the GMO itself.

61. The nature of the activity will also affect the level of risk. Therefore, it is important to take into account the nature of the work or any non-standard operations that might increase the likelihood of release or risk of exposure. For example:

- large-scale manufacture of a GM plant-derived product. This will often mean that large amounts of the GMO will be handled, which may result in increased likelihood of release and exposure;
- the use of non-standard growth facilities. This could be any facility that differs from the usual ‘glasshouse-style’ plant growth structures. For example, this might include the growth of transgenic duckweed in ponds/tanks or culturing GM microalgae in fermenters. The control measures needed to prevent accidental release or exposure will often differ in these facilities.

62. If any such operations or activities are likely to generate risks that are not accounted for in the minimum containment measures already applied in reaction to the risk assessments for the environment and human health, then additional control measures should be applied. Equally, it may be that as a result of the activity, the nature of a risk that is inherent to the GMO itself is diminished. For example, if GM microalgae are cultured in a sealed system, then exposure to workers might be much less likely. In these cases, certain control measures might not be required.

Assignment of final containment measures

63. Unlike work with GM plant-associated microorganisms, there is no regulatory requirement to set a formal containment level (ie Containment Level 1, 2, 3 or 4) for work with GM plants. A number of containment measures and procedures that can be used to reach the required standards can be found below.

64. The environmental risk assessment will have established the principal control measures needed to keep the GM plant contained. The relevant principles of good occupational safety and hygiene should be sufficient to protect human health. These principles are detailed in Part 3, Section 3.1. However, if the risk assessment for human health has identified hazards, for example allergenicity of the expressed product, then additional controls may be needed to protect the operators. This is most likely to take the form of restricted access, personal protective equipment such as gloves or coveralls and staff training.

A guide to assigning containment levels for activities with GM plants

65. The majority of plant growth facilities used for activities involving plant-associated GMMs are also used for work on GM plants. This means that the facility has to be assigned into one of four containment levels for GMM work, but the applicability of this to working solely with GM plants is not always clear. However, some of the measures appropriate for controlling GMMs may also be applicable to GM plant work, for example, for controlling pollen and seeds.

66. The following guide can be used to determine which containment level is appropriate for certain types of GM plant work. The use of Containment Level 1, 2 or 3 for GM plant work is based the use of ‘standard’ glasshouse or a similar facility. Non-standard growth facilities (for example the growth of lower plants in tanks) may require special consideration and containment measures. It is not envisaged that any plant growth facility in the UK will need to be of Containment Level 4 standard.

67. While there are legal minimum containment requirements for GMMs, no such system exists in the legislation for activities with GM plants, and users may adopt other methods as long as the basic requirements set by the EPA to prevent GM plants from entering the environment and causing harm are met. Therefore, the examples used below are illustrative and are provided for guidance purposes only.

68. ***Containment Level 1.*** A Level 1 facility is appropriate for GMMs in association with plants where there is no risk of harm arising. They may also be appropriate for GM plants which are derived from: (i) exotic, non-indigenous plant species which are unable to survive, establish and disseminate in even the most optimum of receiving environments; or (ii) indigenous species which are capable of surviving and establishing but where the nature of the plant provides a reasonable assurance that there will be no dissemination of pollen or seed during the course of the contained use activity. Level 1 facilities are also suitable for plant cell cultures, provided no pathogens are present and as long as they have not been modified to be potentially harmful to humans.

69. Many activities involving the model plant species *Nicotiana tabacum* would fall into the first category. *N. tabacum* is not widely grown for commercial purposes in the UK, has no known relatives that are native to the UK. It does not cross-pollinate with ornamental *Nicotiana* species grown in the UK. *N. tabacum* seeds may be capable of overwintering in the UK, although their germination is likely to be terminated by spring frosts. Therefore, unless the modification increases the ability of the GM *N. tabacum* to survive, establish and disseminate in the receiving environment, level 1 is likely to be appropriate.

70. An example of the latter case would be activities involving indigenous tree species that will be terminated prior to the trees becoming sexually mature, or activities involving young sugar beet that are terminated prior to the production of a flowering stalk in the second year. If the experiments were completed within the first year and any rogue flower spikes were immediately removed then level 1 may be appropriate, depending on the outcome of the risk assessment.

71. ***Containment Level 2*****.** A Level 2 facility is appropriate for plant-associated GMMs where the risk assessment has identified a low risk of harm should the organism escape. They may also be appropriate for GM plants that are able to survive, establish and disseminate in the receiving environment but have genetic modifications that are unlikely to cause harm to either humans or the environment, as defined by the EPA. For example, many activities involving the model plant species *Arabidopsis thaliana* are likely to fall into this category of containment. *A. thaliana* is used in many GM centres for fundamental genetic studies and individual GM activities may result in many thousands of seeds being produced which are capable of surviving in the receiving environment, establishing populations and disseminating the transgenes in the environment. However, the vast majority of modifications involving *A. thaliana* are unlikely to result in a more harmful phenotype and Containment Level 2 measures are considered reasonably practicable to limit contact with both humans and the environment to an acceptably low level.

Pollen control measures

P15. Reproductive isolation from sexually compatible relatives in the receiving environment can be achieved by the removal of flowers or halting experiments prior to flowering. Consideration may also be given to the use of male sterile lines or the use of transgene localisation within chloroplasts that may reduce the likelihood of transgene spread through pollen.

P16. Spatial isolation from sexually compatible relatives in the receiving environment can be achieved by ensuring that such plants are a suitable distance away from the facility.

P17. Temporal isolation from sexually compatible relatives in the receiving environment can be achieved by allowing the experimental plants to flower out of the normal season, for example by undertaking the activities in winter.

P18. Physical isolation and reproductive containment is sometimes possible by bagging flower heads prior to anthesis using paper or glassine bags. Alternatively, plants may be contained within secondary insect- or pollen-proof containers. Exhaust air from the facility should be filtered if pollen could be harmful to human health or the environment.

Seed control measures

P19. Spatial isolation from suitable seed germination sites in the receiving environment can be achieved by ensuring such plants are a suitable distance away.

P20. Temporal isolation from suitable seed germination sites in the receiving environment can be achieved by growing experimental plants out of season.

P21. 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. Local rules should be in place detailing the method for seed harvesting and collection.

P22. Sticky floor mats at the exit of the facility can be used to minimise seed dissemination on the feet of staff.

Example GM risk assessments

The following risk assessments give an example format and are for illustrative purposes only. They are not intended to prescribe how GM risk assessments are to be carried out. Furthermore, they are not exhaustive and under each section advice is given on the type of information that would need to be included to provide a comprehensive document that should enable a reviewer (GMSC or external) to determine whether the risk assessment is suitable and sufficient.

Example GM risk assessment: Analysis of pollen-specific promoters in *Arabidopsis*

Overview

The aim of the project is to analyse pollen-specific promoters in *Arabidopsis thaliana* using resistance to the herbicide BASTA as a marker.

An amount of background information regarding the purpose of the work should be included. For example, this study is likely to be basic research into the molecular mechanisms of pollen development. However, if there is a longer-term aim for the work, such as the development of a novel biological containment approach, it would be helpful to state this here to inform the risk assessment.

Risk assessment for the environment

Mechanisms by which the GMO might pose a hazard to the environment

Can the GMO survive, establish and disseminate?

A. thaliana is a native to the UK and will survive and disseminate within the receiving environment.

The risk assessment would benefit from further information regarding the nature of the host plant. For instance, the cultivar to be used is Landsberg *erecta*, which tends to favour laboratory cultivation. Furthermore, the literature indicates that these cultivars will not be able to compete with other species in the UK environment (although this data is limited). The section should include a discussion as to whether or not planned modifications are likely to change these characteristics.

What hazards does the inserted material pose?

The inserted genes encode for *bar*, which in turn confers resistance to the herbicide BASTA.

Relevant facts regarding the insert and expression characteristics should be included here. For example, details of the gene products' mechanism of action could be outlined. Furthermore, the fact that this gene has been widely used in plant transformation studies for many years without reports of harm is important information.

Could the GMO or other organisms acquire harmful sequences?

Acquisition of harmful sequences is unlikely as *A. thaliana* is self-compatible and predominantly inbreeding.

All possible mechanisms of sequence acquisition should be assessed. For instance, cross-pollination via insect vectors or the airborne route is a possibility, although considered to be a rare event restricted to plants in close proximity. The likely use of BASTA as a herbicide in the environment and the potential consequences of the spread of the resistance gene should be considered.

Is the GMO phenotypically/genetically stable?

Yes.

It would be helpful to qualify this assertion. For instance, since the inserted genes encode a herbicide resistance marker, the GMO will be genetically stable in the laboratory where the herbicide will be used. However, in the environment it is unlikely that there will be selection pressure in favour of retaining the inserted gene, unless the herbicide is widely used. Therefore, it is possible that the gene will be lost in the event that the plant establishes itself in the receiving environment.

Likelihood that GMO will be a risk to the environment

What is the likelihood that the hazard(s) will be manifested?

In the event that the GMO is inadvertently released into the environment, via seed or pollen, it is likely that germination will occur, or that cross-pollination with wild-type relatives may take place. The likelihood of these adverse events occurring can be considered to be **medium**.

How severe might the consequences be?

If seeds of the GM plants were to enter the environment, the resulting plants will be capable of surviving and disseminating. However, these plants are not expected to be able to compete with environmental species, unless they are exposed to BASTA in the environment. Furthermore, given the limited hazards posed by the inserts, should this occur the consequences are considered to be **minor**.

The level of detail required in this section will vary depending upon the nature of the GMO and hazard it poses. This is why the characteristics of the recipient organism and the inserted material must be explained in detail above to aid justification of the assertions made here. If plants cannot disseminate in the receiving environment, little detail will be required, but where there is greater degree of uncertainty a more extensive reasoned argument should be included.

Determine risk level to the environment

Using the risk determination matrix, the risk to environment is **low**.

Containment measures needed to protect the environment

Suitable control measures for pollen and seeds will be in place. These will reduce the risk to the environment to **effectively zero**.

Details of the measures needed should be included here. For instance, flower heads may be bagged in order to control pollen dissemination and limit spillage of seed. The drainage system may also be filtered and all plant material autoclaved. Strict hygiene measures should be in place to collect seeds and control them spilling onto the floor and staff should be appropriately trained.

Risk assessment for human health

For most work with non-food GM plants, the risks to human health will be low, unless there is expression of a product that is potentially toxic or allergenic.

Mechanisms by which the GMO might pose a hazard to health

Are there any health hazards associated with the GMO?

No – *Arabidopsis* is not a plant that is consumed by humans, so the likelihood of ingestion is low. The modifications are not expected to result in an increase in the hazards posed by handling plant material or exposure to pollen.

What hazards does the inserted genetic material pose?

Selection using *bar* has been widely employed in plant transformation studies for many years without reports of harm. Furthermore there are no known toxic effects attributed to the gene product or its action.

Likelihood that GMO will be a risk to human health

Likelihood of hazards being manifested

The likelihood that harm to humans will arise following exposure to the GMO is **negligible**.

How severe might the consequences be?

Even if plants were to be accidentally consumed, no harmful effects are known of or anticipated. Therefore, the consequences are considered to be **negligible**.

Determination of risk to human health

Using the matrix, the risk to human health is **effectively zero**.

Containment measures needed to protect human health

No new measures in addition to those measures used to protect the environment are needed.

Review procedures and control measures

Implement measures to safeguard human health and the environment

Are there any non-standard operations that might increase risk?

No.

What control measures and monitoring procedures are to be used?

Standard good practice in a glasshouse facility should be sufficient. The risks to both environment and human health are **effectively zero** so no extra control measures are required.

It would be helpful to list or refer to the final range of measures that are being employed in this section.

Are the potential routes of environmental release known and managed?

The most likely routes for the release of the GMO into the environment are via seed and pollen dispersal. These routes are known and managed.

Notification requirements

The GMO will not represent an increased risk to human health. No notification required.

Example GM risk assessment: Expression of peptides in plants using a plant virus

Overview

The aim of the project is to express the human endostatin peptide in the plant species *Nicotiana benthamiana* using Potato virus X (PVX).

An amount of background information regarding the purpose of the work should be included. For example, the longer-term aim for the work might be to develop a system for production and manufacture of a therapeutic product, which would be handled in large numbers and possibly marketed. It would be helpful to state this here to inform the risk assessment.

Risk assessment for the environment

Mechanisms by which the GMM might pose a hazard to the environment

Can the GMO survive, establish and disseminate?

PVX occurs naturally in the UK, causing disease in potatoes. The recipient strains are naturally occurring UK field isolates. The burden of the inserts is likely to reduce the fitness of the GMM in the wider environment, and it is anticipated that the inserts will be rapidly lost. Therefore, it will be assumed that the GMMs constructed will retain the ability to establish infections in the UK plant hosts.

Further information about the nature of the recipient strain should be included, for example its host range, properties of transmission and mechanisms of spread. Statements regarding fitness and the potential loss of inserts must be qualified, perhaps by using references to scientific data and the literature. Where there is uncertainty, a precautionary approach should always be taken. For work like this that involves novel methods for the production of pharmaceutically active products, the regulatory authorities will require greater detailed evidence regarding the safety of the GMM.

What hazards does the inserted material pose?

The insert will encode human endostatin peptide, which is normally produced in humans and animals during wound healing. The gene will be expressed at high levels in plants via a duplicated subgenomic promoter for the PVX coat protein. The plant material will not be consumed by humans or animals in the laboratory and, as such, is not anticipated to pose a hazard.

The expressed product would not normally be present in the receiving environment in the context of the GMM or infected plant. Once again, a precautionary approach must be taken, as there is unlikely to be substantial evidence of how this product will affect the environment. Any assertions as to the safety of the product in the environment will need to be justified and are likely to be closely scrutinised.

Have the pathogenic traits of the recipient strain been altered?

The expression of the peptide is not anticipated to alter the pathogenicity of the virus, or its routes of transmission. If the virus was to escape and infect plants, endostatin could be expressed in the field. It is not expected that this in itself will be harmful.

These statements would need to be fully justified, using a reasoned argument. The regulators would not accept simple statements such as ‘it is not anticipated or ‘is not expected to be harmful’ without proper justification and supporting evidence.

Could the GMM or other organisms acquire harmful sequences?

No.

Further details and justification for this answer should be included here. For example information about the potential for PVX to recombine with viruses in the field or stable transfer of the inserted sequence to the plant genome would be expected.

Is the GMM phenotypically/genetically stable?

Yes.

Statements like this must be justified. In this case it is hard to justify the statement as arguments have already been presented that the insert will be rapidly lost from the virus. Therefore, the GMM is not genetically stable, even if the consequences of such an event are considered to be negligible.

Likelihood that GMM will be a risk to the environment

What is the likelihood that the hazard(s) will be manifested?

There are no intermediate vectors (eg arthropods) known for PVX and the main route of environmental exposure is likely to be mechanical transmission via infected plant material. Given that host plants are not grown in the vicinity of the facility, the likelihood of the GMM escaping and infecting potato plants is **low**.

Clearly, the likelihood on environmental release and dissemination of the GMM will be much higher if host plants are grown commercially or privately in the immediate environs of the facility. This is unlikely if the facility is in an urban area, but the likelihood of escape and dissemination will be higher in a rural setting.

How severe might the consequences be?

Should the GMM escape and find a suitable host, it is assumed that they will be able to initiate an infection and express endostatins in plants. While it is not expected that the disease symptoms elicited by the GMM will be any different from those associated with the wild-type organism, a novel protein will be expressed. Therefore, the consequences can be considered to be **modest**.

Given that the expressed product will be novel in the context of the GMM or host plant, there is a high degree of uncertainty and a precautionary approach should always be taken.

Determine risk level to the environment

Using the determination matrix, the risk to environment is **medium/low**.

Containment level needed to protect the environment

All laboratory work will be undertaken at Containment Level 2. This will reduce the risks to the environment to **effectively zero**.

A brief explanation as to why Containment Level 2 is appropriate for this work and what specific measures are to be used should be included.

Risk assessment for human health

Mechanisms by which the GMM might pose a hazard to health

Are there any health hazards associated with the GMM?

Endostatin is naturally occurring in the human body. It will be expressed to high levels in experimental plants, but these will not be consumed. It is not anticipated that exposure to the peptides in the sap of infected plants will increase the allergenic or toxic hazards associated with the plants.

These statements would need to be fully justified, using a reasoned argument. The regulators would not accept simple statements such as ‘it is not anticipated’ without proper justification and supporting evidence. As endostatins are in therapeutic use, information on the toxicology of the product should be readily available.

Likelihood that GMM will be a risk to human health

Likelihood of hazards being manifested

Likelihood that humans will be exposed to hazards associated with the GMM is **low**.

Justification for this assertion is required and will depend upon the likely route of exposure. For example, if the product is a potential allergen and humans may be exposed through handling the plants, then specific control measures (eg gloves, coveralls) may need to be assigned below.

How severe might the consequences be?

Even if humans were to be exposed, no harmful effects are anticipated. Therefore, the consequences of exposure are considered to be **negligible**.

A reasoned argument as to why there are no anticipated harmful effects is required here and will depend upon the toxicology of the product.

Determination of risk to human health

Using the determination matrix, the risks to human health are **effectively zero**.

Containment level needed to protect human health

As no harmful effects are anticipated in the event of exposure, Containment Level 1 would be sufficient.

The use of protective clothing or gloves may be indicated as part of the environmental risk assessment to prevent release of the GMM into the environment. Such measures may be sufficient to protect against worker exposure, however, if they are actively required for this purpose, then Containment Level 2 is appropriate for protection of human health.

Review procedures and control measures

Implement measures to safeguard human health and the environment

Are there any non-standard operations that might increase risk?

No.

What control measures and monitoring procedures are to be used?

Measures are in implemented for environmental protection. Standard good practice in a Containment Level 2 glasshouse facility will be sufficient, including measures to control mechanical transmission of the GMM.

Details of the control measures used should be included. For example, the plants will be grown in pots and stored on trays within a locked room in a glasshouse for three weeks before being harvested. During this time only trained, authorised operatives will enter the facility. All watering will be via a watering can, taking care being not spread the virus between plants. All infected waste (including plants, pots and soil) will be autoclaved and strict hygiene measures will be observed in the growth room, including the wearing of gloves which will be disposed of through autoclaving and removal.

Are the potential routes of environmental release known and managed?

The most likely routes for release of the virus into the environment are via contaminated waste plant material and human mechanical transmission. These routes are known and managed.

GM activity classification (Class 1, 2, 3 or 4)

The Containment Level 2 measures described above for environmental protection are considered appropriate for ensuring that all risks are **effectively zero**.

The activity is therefore assigned to GM Class 2.

Further information

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