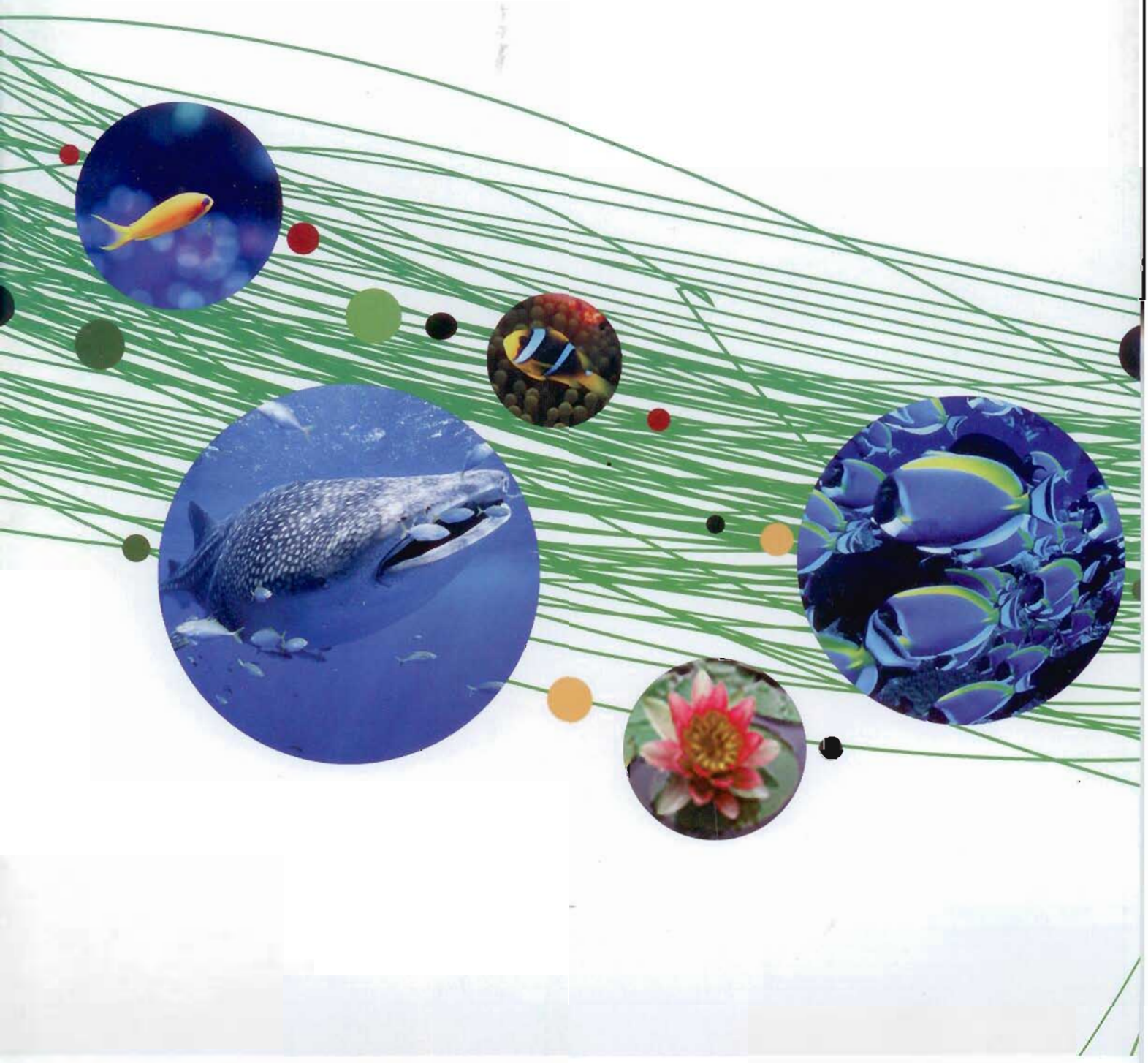
RISK ANALYSIS CONTAINED USE RESEARCH AND DEVELOPEMENT ACTIVITIES WITH GENETICALLY MODIFIED AQUATIC ORGANISMS

GUIDANCE DOCUMENT



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Part 1: Background

I. Introduction

Genetically modified (GM) aquatic organisms are defined as aquatic organisms, including protozoa, algae, plants and invertebrate and vertebrate animals, of which the genetic material has been modified in a way that does not occur naturally through mating or natural recombination or both. These guidelines are proposed for use during the design, registration and management of physically contained research and development (R&D) facilities for GM aquatic organisms in South Africa. This includes pilot plants, in which case the containment measures should correlate with the potential risk of exposure, but excludes commercial production activities with aquatic GMOs.

Although this document proposes a number of measures for the design, registration and management of R&D facilities this is currently a guidance document only and has no regulatory standing (specifically under the GMO Act (Act No. 15 of 1997) and the GMO Act Amendment Act (Act No. 23 of 2006) or their associated regulations or formal guidance documents). It is proposed, however, that a guidance document specifically focusing on contained GM aquaculture facilities should be formally integrated into the regulatory system, which governs the use of GMOs in South Africa, and this document may be used as a basis for such a document.

Complete containment often implies prevention of escape of all the organisms within a facility whereas confinement generally refers to reducing the likelihood of escape from the facility or site. Within the South African regulatory system with regards to GMOs containment refers to the prevention of escape from a contained facility (for work conducted in a CL1-4 contained use facility). The risk assessment procedure includes the classification of activities into one of four containment levels. Containment measures form a continuum rather than four discrete levels and activities may require control measures that are a combination of features from different containment levels. Any facility in which activities with GMOs take place is required to be registered and activities within that facility require a permit. Accordingly, the term 'containment' as opposed to 'confinement' is employed in this document. When applied to current procedures used to regulate GM plants this is analogous to contained use in a lab and/ or greenhouse or other suitable facility. The use of the term confined use may be more applicable to pre commercialisation, larger scale activities analogous to confined field trials and commercial environmental release.

Although, activities with aquatic GMOs in academic and research facilities may be exempt from permit applications, as stated by regulation 2(2) of the GMO Act, 1997 (Act No. 15 of 1997) and the 2005 standard operating procedures with regard to regulation 2(2) of this Act, it is suggested that no distinction is made between academic and any other R&D facilities in this instance. Moreover, it is proposed that the current DAFF documents "Application to register a facility for activities involving genetic modification" and *"Application for contained use of genetically modified organisms (GMO's) in South Africa" are replaced* the intended activities or the organism from other relevant legislation, by a new document "Application to register a facility for R&D activities involving genetically modified aquatic organisms" (attached hereto), for all R&D activities in South Africa involving GM aquatic organisms. This document deals specifically with R&D facilities as this aligns with the current regulations governing contained use activities in South Africa (i.e. the registration of a contained use facility and a permit for activities in a contained use facility). Another document covering the process for licenses for larger scale pilot plants and commercial production systems in confined or unconfined facilities can be developed at a later stage (perhaps when more experience is gained with aquatic containment systems or as the need arises).

II. Compliance with all relevant legislation

If an organism, irrespective of the fact that it may be GM, has not previously been released into a particular environment it will be subject to various pieces of legislation, which aims to reduce the risk of introducing an organism with unwanted negative effects into said environment (for a comprehensive overview with regards to the environmental risk assessment of aquatic GMOs see Kapuscinski et al 2007). If GM aquatic organisms are imported into South Africa they will also be subject to international agreements such as the Cartagena Protocol on Biosafety (http://bch.cbd.int/protocol) and possibly, if the organism is intended to be used as a food, the Codex Alimentarius (http://www.codexalimentarius.net/web/index_en.jsp). In addition, local biosecurity legislation and international guidelines aimed at preventing the introduction of new pests and diseases will be applicable. Relevant local legislation to consider includes the National Environmental Management Act (No. 107 of 1998), the National Environmental Management Biodiversity Act (No. 10 of 2004), and the Conservation of Agricultural Resources Act (No. 43 of 1983), the Agricultural Pests Act (No. 36 of 1983), the Animal Improvement Act (No. 62 of 1998) and the Marine Living Resources Act (No. 18 of 1998). International guidelines on the transfer and introduction of aquatic species include the Food and Agriculture Organization (FAO) of the United Nations' "FAO Technical Guidelines for Responsible Fisheries: Aquaculture Development – 5" (FAO, 1997) and the International Council for the Exploration of the Sea's (ICES) "ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005" (ICES, 2005).

The number and apparent complexity of these regulatory hurdles could translate into an insurmountable legislative burden, which could impede on the development and utilisation of GM technologies in aquaculture, if it is not sensibly rationalised and managed. Both regulators and technology developers should therefore endeavor to identify commonalities, exceptions and possible conflicts in order to establish an amenable, efficient and effective regulatory framework for aquatic GMOs. This is feasible because all these regulations and guidelines share the principle requirement for a comprehensive risk analysis, which would allow the sharing of information and a complementary approach.

To prevent unnecessary duplication and conflict it is therefore envisioned that the potential environmental, evaluated and managed under the GMO Act. However, this will not absolve e.g. adherence to a health certification scheme and/or a pathogen

risk assessment based on the guidelines of the World Organisation for Animal Health (OIE), as required by the relevant regulators. Finally, when GM aquatic organisms are specifically considered the characteristics of the "entire" GMO, including any possible risk mitigation features, should be considered when doing the risk analysis. For example, the GM aquatic organism may contain additional traits (including GM traits) to decrease its potential environmental impacts, such as biological containment or pathogen resistance traits.

III. Risk Analysis of GM aquatic organisms

Risk analyses of GM aquatic organisms will be conducted as for any other GMOs and include three activities, i.e. risk assessment, risk management and risk communication. (i) Risk assessment consists of two distinct actions, i.e. hazard identification, and risk characterisation, which includes the assessment of likelihood and consequence of harms and an estimation of the level of risk. During these steps plausible pathways to harm are identified and the levels of risk associated with particular hazards are estimated (discussed in more detail below). (ii) Risk management is the process whereby appropriate strategies are selected and implemented to ensure that possible risks are appropriately reduced and managed to ensure that activities involving the GMO take place at an acceptable level of risk. (iii) Risk communication is the flow of information and substantiated views between all the stakeholders, e.g. between the Scientific Advisory Committee (risk assessors) and the Executive Council (regulatory decision maker) of the GMO Act and between regulators and other stakeholders, including, for example, regulatory decisions and the rationale for making them.

The purpose of risk assessment in a GM research and development program is to proactively identify plausible hazards and characterise possible risks to human health/safety and the environment to enable decisions with regard to allowing the activity to proceed and to determine appropriate risk management (e.g. containment measures). Hazards (acts or phenomena that have the potential to cause harm) associated with a GMO are identified by postulating plausible pathways that would lead to harms. Harms are undesirable consequences to humans and things that they value (these harms to human health and/or the environment must be clearly defined). The level of risk associated with a particular hazard is estimated by evaluating the likelihood and potential consequence (magnitude) of the harm occurring.

Note: Information requested in the "Application to Register a Facility for Research and Development Activities Involving Genetically Modified Aquatic Organisms" covers a broad range of topics to enable the regulators to identify potential harms and characterise potential risks that the applicant may not have considered.

Identifying risk, 2010) can ensure that only information relevant to risk analysis and regulatory decision making is scenarios that postulate plausible clearly defined pathways to harm (Raybould 2006 provided and/or generated. If well designed this can lead to minimising information unnecessary for effective risk analysis.

Part 2: Guidelines for the risk analysis of contained use R&D activities withGM aquatic organisms

As the unintentional escape of GM aquatic organisms is a crucial step in the pathway to the majority of plausible harms associated with GM aquatic organisms, containment can effectively negate these associated hazards. The focus of a risk analysis exercise for R&D activities within a contained use facility should therefore focus on the risk mitigation measures, most importantly physical containment measures, to ensure all possible risks are reduced to acceptable, low levels. Other containment methods such as biological containment and geographic containment can also be considered.

Guidelines have been developed for the physical containment of GM aquatic organisms that take various life history traits and the characteristics conferred by the transgenes into account (ABRAC, 1995; Scientists Working Group on Biosafety, 1998). The former document is a useful practical tool when establishing adequate containment and management strategies for a contained aquatic GMO facility. The above mentioned guidelines contain a framework for systematically evaluating the activities with GM aquatic organisms. These assessments consist of flowcharts that can guide the consideration of the potential hazards associated with the particular activity and assist with regards to appropriate mitigation and containment measures that can be applied in a contained use aquaculture facility. Decision making pathways as outlined in these guidelines can be provided in Section 2.3 of the application document to support the risk mitigation procedures that are proposed by the applicant.

I. Risk assessment

The primary objective of a risk assessment for the activities with a particular GM aquatic organism is to determine the likelihood and potential consequences of the identified harms associated with these activities. Assessing the potential human health/safety and environmental risks of a particular activity is therefore based on two consecutive steps, i.e. hazard identification and risk characterisation.

Hazard identification

The objective of this first step in the risk assessment process is to identify plausible pathways that may lead to harm (risk scenarios). To facilitate this information is required with regards to:

- the characteristics of the GM organism itself, e.g. its motility, resilience and size.
- the new genetic insert, i.e. the transgene(s) and regula tory sequences, and the traits they encode, e.g. enhanced growth and disease resistance.
- the receiving environment, e.g. the presence of native populations with which the GMO can interbreed resulting in ecological harm.

Although many apparent hazards may be identifiable from these individual sources, an integrated analysis is necessary to establish which pathways to harm are plausible under a specific set of conditions. For example, the potential for gene flow between the GM aquatic organism and other organisms will depend on the characteristics of both the organisms and the receiving environment, while the consequences thereof will also depend on the GM trait. One of the values of the hazard identification process lies in the fact that appropriate risk management practices can be identified to ensure possible pathways to harm are disrupted. For example if a GM aquatic organism has the potential to cause a negative ecological impact (e.g. it is likely to interbreed with a closely related indigenous species which may lead to a decrease in the population size of the indigenous species), sufficient containment measures should be put in place to ensure the overall level of risk is acceptably low.

Information important to collect during this phase includes, but is not limited to, the following:

i) Information related to the GM aquatic organism.
ii) Information on the receiving environment.
iii) Information related to the transgene in the GM aquatic organism.
iv) Information related to the interactions between the GMO and its environment.
v) Potential impact on the immediate environment if there is an accidental breach in containment.
vi) Human health and food safety aspects.

Risk characterisation

Subsequently, the risk associated with each identified hazard must be assessed in order to establish appropriate containment measure for the activities. The level of risk is estimated based on the likelihood and consequence of the harm. This risk estimate is the product of the likelihood of a harm occurring and the potential consequence if it occurs and can be based on a risk assessment matrix (Fig 1 and Tables 1 & 2). Although risk assessment matrixes are valuable tools to help estimate relative levels of risk, they do not give a definitive measure of risk. Assessing both the likelihood of an occurrence and the consequence of the occurrence is open to interpretation by suitably qualified persons, based on the information which is available at that point in time. Moreover, in many R&D projects only limited information will be available on the possible consequences of any particular risk scenario. When uncertainty exists focus can be placed on containment strategies to ensure the likelihood of harm is decreased to a level where it will decrease the level of risk to acceptably low levels. There are different types of uncertainty that can be encountered during risk analysis. These include uncertainties in or gaps in knowledge, variability, descriptive uncertainty and cognitive uncertainty. These uncertainties must be recognized and where possible reduced or managed. Uncertainty is particularly important to consider during risk characterisation. Risk management is often used to deal with uncertainties in the risk analysis process.

In a contained use facility the focus is on containment to reduce the likelihood of harms (in the majority of risk scenarios the escape of aquatic organism will be a crucial step in the pathway to harm).

Figure 1. Risk assessment matrix (from "Risk Analysis Framework April 2009", OGTR, 2009).

		RISK ESTIMATE			
LIKELIHOOD	Highly likely	Low	Moderate	High	High
	Likely	Low	Low	Moderate	High
	Unlikely	Negligible	Low	Moderate	Moderate
	Highly unlikely	Negligible	Negligible	Low	Moderate
		Marginal	Minor	Intermediate	Major
		CONSEQUENCE ASSESSMENT			

Table 1. Scale for the level of risk (from "Risk Analysis Framework April 2009", OGTR, 2009).

Risk estimate	Risk estimate definitions
Negligible	Risk is insubstantial and there is no present need to invoke actions for mitigation.
Low	Risk is minimal, but may invoke actions for mitigation beyond normal practices.
Moderate	Risk is of marked concern that will necessitate actions for mitigation that need to be demonstrated as effective.
High	Risk is unacceptable unless actions for mitigation are highly feasible and effective.

Table 2. Scale for assessing likelihood and consequence assessments (adapted from "Risk Analysis Framework April 2009", OGTR, 2009).

Likelihood[1]	Likelihood assessment definition[1]
Highly unlikely	May only occur in very rare circumstances
Unlikely	Could occur in some circumstances
Likely	Could occur in many circumstances
Highly likely	Is expected to occur in most circumstances
Consequence[2]	Consequence assessment definition (related to human health and environment)
Marginal	No or minimal adverse health effects or damage/disruption to the environment
Minor	Adverse but limited and reversible health effects or damage/disruption to the environment that is reversible and limited in time, space and numbers affected
Intermediate	Adverse, widespread and not readily reversible health effects or widespread damage/disruption to the environment that is of limited severity and reversible
Major	Adverse, severe, widespread and irreversible health effects or extensive damage/disruption to whole natural ecosystems, communities or species that persists over time and is not readily reversible

[1]Quantitative values to better define these qualitative terms could be determined on a case-by-case basis by the relevant stakeholders.
[2]In South Africa economic considerations should be incorporated into the likely consequence assessment.

Level of detail required for a risk assessment

The required level of detail will vary depending on the extent of the identified risks and the degree of uncertainty, i.e. uncertainty would merit more detailed discussions and supportive data. In general, the risk assessment must contain sufficient information to enable a reviewer to understand the nature of the risks without requiring further information. Supplementary information in the form of references of scientific literature and reports may be provided as annexes to the application.

A risk assessment must be reviewed as new relevant scientific knowledge becomes available or where there is a change in the activity with an aquatic GMO. This includes a change in the scale of the activity, procedures used and containment measures. More information with regards to this can be found below.

II. Risk management: Containment of GM aquatic organisms

Risk management is the step following risk assessment where strategies are adopted to ensure that risks are appropriately managed. Risk mitigation is considered during risk management for risks that are estimated to be greater than negligible. If risks posed by activities with GMOs cannot be managed/mitigated so that activities can take place in an acceptable level of risk then a permit for that activity will not be issued.

Containment measures are often the employed during risk management to mitigate identified risks. The main aim of these guidelines is to help ensure the adequate containment of GM aquatic organisms used in R&D projects. Contained use as defined in section 1 (v) of the GMO Act (No. 15 of 1997) means:

> *"Any activity in which organisms are genetically modified or in which such genetically modified organisms are cultured, stored, used, transported, destroyed or disposed of and for which physical barriers or a combination of physical barriers together with chemical or biological barriers or both are used to limit contact thereof with the environment."*

Aquatic organisms pose particular issues regarding their containment and likelihood of establishment in the environment. The risk of escape and establishment of these organisms could be relatively high because of a number of factors, including:

- Low levels of domestication in these organisms-animals that tend to easily become feral pose the greatest concern with regards to GM animals. The more domesticated a species is the less likely it is to survive in natural environments (NRC, 2002);
- Aquaculture production systems are often located in areas where conspecifics or closely related species occur and gene flow can therefore take place;
- Aquatic organisms often have high dispersal abilities that must be considered when implementing containment systems. Larval stages not only pose certain physical containment challenges but, additionally, often give these organisms the potential to disperse quickly over large distances (ABRAC, 1995). In addition, reproductive strategies of aquatic organisms often rely on the release of large numbers of fertile eggs into the environment;
- Aquatic organisms are often cultured and marketed live (NRC, 2002).

Containment methods

There are a wide variety of containment measures that may be employed to minimise the likelihood of escape of GM aquatic organisms into the environment. These can be grouped into three main containment strategies, i.e. physical (including physicochemical), biological and geographical. There are a variety of different biological, mechanical and physical containment measures which can be applied to GM aquatic organisms in different aquaculture systems (see Van Eenennaam and Olin 2007 for systems developed for GM fish). The containment strategies and measures employed in a GM aquatic organism facility may vary widely to take into account factors such as the characteristics of the organisms, the traits expressed and the potential receiving environments. The containment methods used must also be appropriate for the containment facility (e.g. closed land based systems vs. open systems).

Physical containment is the first line of defence in preventing aquatic GMOs and viable gametes from escaping into natural habitats. Guidelines on the physical containment of transgenic aquatic organisms have already been developed (ABRAC, 1995; Scientist's Working Group on Biosafety, 1998). The focus of these established guidelines is on the containment of GM aquatic organisms within research facilities. This physical containment should be integrated with effective management strategies to prevent the escape of aquatic GMOs. These measures could also be applied/adapted as necessary for commercial production systems, depending on the level of risk identified during the risk assessment process for commercial production. However, the efficacy of these systems should be confirmed during the R&D phase.

Physical containment methods include systems such as mechanical barriers or involve effluent water treatment to ensure that the water chemistry is lethal to different life stages of the aquatic GMO (including gametes). Mechanical barriers block the passage of one or more life stages of the aquatic organism and often consist of filters or screens. The use of temperature changes, changes in the pH, radiation or biocidal agents to the effluent water flow are examples where the physical and chemical conditions are manipulated to induce mortality and prevent escape.

The design of a system in which physicochemical barriers are in use would normally consist of a chamber in which the lethal condition or conditions are imposed, followed by a chamber in which the water condition is returned to normal before the effluent is discharged into the water body. This is done so as not to negatively influence the ambient environmental conditions in the natural water body into which the effluent is discharged. Physical containment systems must be able to be effective in containing the escape of even the smallest form in the life cycle of the aquatic organism, bearing in mind that gametes or asexual propagules of aquatic organisms can be very small, for example, viable fish eggs or newly fertilized embryos can be 10 µm (NRC 2004).

Other possible methods to reduce potential environmental risks include biological containment methods and geographical containment. Biological containment aims to block reproduction and constrain gene flow and persistence in the environment. Examples of biological containment include the introduction of sterility in the transgenic aquatic organism, which is to be contained (NRC, 2004; Uzbekova et al. 2000; Maclean et al. 2002; Fu et al. 2005; Slanchev et al. 2005). Sterility can be accomplished by non-transgenic means, such as the induction of triploidy, or by transgenic means such as genetic use restriction technologies (GURTs). Other possibilities for biological containment include the development of a single sex population, for example an all female transgenic line where the females are fertilized with cryopreserved sperm of a compatible species. This will result in sterile transgenic hybrids (Le Curieux- Belfond et al. 2009).

Geographical containment entails situating a GM aquatic organism facility in a site where the environmental parameters fall outside of those suitable for the survival of the organism or establishing facilities away from natural or closely related populations. Independently or in combination these measures will therefore help ensure that a GMO will not be able to survive and reproduce in the environment if it escapes. This would reduce many of he risks of aquatic GM organisms to the environment and human health.

Risk mitigation features for contained use activities with a GM aquatic organism during R&D focuses on the physical (including physicochemical measures) and geographical containment of GM aquatic organisms but biological containment methods could also be considered to ensure suitable containment. However, although biological containment approaches are not required by regulators for activities during the R&D stage, biological containment methods will be beneficial and might even be essential when considering/applying for the large scale/commercial use of GM aquatic organisms.

Factors that influence containment

No universal guidelines for the containment of GM aquatic organisms currently exist due to the large variability in scale, locations, organism characteristics, possible effect conferred by the GM trait and facility design. Moreover, the containment of GM aquatic organisms in a contained use facility increases in difficulty (with corresponding difficulties in maintaining effectiveness) with a number of factors, which includes:

- Decreasing size of the GMO (with consideration of differ ent life stages) and its propagules.
- Increasing fitness and physical tolerance of the organism.
- Increase in dispersal ability of the organism and its propagules.
- Increase in the scale of the activity – including the duration, the number of organisms contained, the amount of waste and/or effluent produced and the physical size of the facility.
- Proximity to receiving environments that assist dispersal, for example the sea; rivers, drainage systems and other water bodies.

Additionally, growing aquatic organisms in land-based systems as opposed to non-land based systems (pens, cages, off-bottom and on bottom culture) is likely to minimise the potential escape of the aquatic organism into the wild. Appropriate containment measures must be implemented to ensure the suitable containment of the transgenic aquatic organism taking into account the potential likelihood of escape and potential harm associated with that escape. Containment strategies must therefore be effective at ensuring that the overall level of risk is low or negligible.

Scale of the activity

The scale of an activity with a GM aquatic organism influences the level of containment because it could impact on both the likelihood and consequence of a potential breach in containment. The scale of the activity is determined by the physical size (area), the amounts of waste produced, the volumes of effluent contained and discharged, and the number of organisms contained in the facility. This would imply that if, for example, a growth facility's physical size is increased or there is an increase in the volume of effluent produced, or if the activity moves from laboratory work to also include dedicated growth facilities (as outlined below) a new application for certification as a Physical Containment Aquatic Organism Facility will be required.

Based on this two different facility categories can be envisaged for contained R&D activities:

1. R&D aquaculture laboratory facilities: Laboratory based research within a fully enclosed, solid structure/building, involving small scale activities with GM aquatic organisms. Small aquariums are within or directly linked to the laboratory. Only very small volumes of waste and/or effluent are produced. Typically such research will be done to optimise transformation procedures, perform basic molecular genetic analyses and the initial analysis of a GMO's physiological performance. These facilities will typically produce effluent/waste in batches or very small amounts of effluent from continuous, flow through systems. The suggested limits for R&D laboratory facilities are:

- Physical size of the facility: ≤ 100 m2.
- Total contained volume: ≤ 1,000 litres.
- Average waste1 volume per day: ≤ 1 litre (or kg)/day.
- Average batch effluent2 per day: ≤ 100 litres/day.
- Average flow through effluent per day: ≤ 200 litres/day.

1 Waste is defined as any material, used during any activities with the GMO, which is likely to contain GM material or organisms and that is likely to be discarded in batches, e.g. lab consumables, genetic material, sacrificed cells or whole organisms and filtered material.

2 Effluent is defined as the water, which is discharged from an aquaculture system/aquarium into a drainage system.

2) R&D aquaculture facilities: Small and medium scale R&D facilities which could include both laboratory and associated growth facilities and where the growth facilities are not necessarily fully enclosed within a solid structure. Typically these facilities will do later stage R&D work which focuses on the detailed characterisation of GMOs. These may, for example, include pilot plant scale activities to simulate conditions that are more realistic to those found in production facilities. The suggested limits for R&D aquaculture facilities are:

Physical size of the facility:	≤ 2,500 m2.
Total contained volume:	≤ 50,000 litres.
Average waste volume per day:	≤ 50 litres (or kg)/day.
Average batch effluent per day:	≤ 5,000 litres/day.
Average flow through effluent per day:	≤ 10,000 litres/day.

Because aquatic organisms represent a wide variety of organisms and production systems it is possible that the above scale definitions will have to be tailored under certain conditions. In cases where the scale of bona fide R&D activities (i.e. not commercial aquaculture) may exceed the specified limits set above the following should be comprehensively motivated in the application:

- Constraints in keeping within the size, total and effluent/ waste volumes of the above guidelines.
- How the suggested increased scale of activities is applicable to either a R&D aquaculture laboratory or a R&D aquaculture facility.
- How containment measures will adequately consider the proposed scale of the activity.

Although these guidelines only apply to small and medium scale R&D activities with aquatic GMOs, containment methods designed and implemented during this stage should be used as a basis when designing relevant containment systems for the possible commercial production of the GM aquatic organism under contained conditions. Finally, some R&D and even commercial production systems may not require contained conditions, but a decision can only be made on a case-by-case basis after a relevant risk assessment of the activity with a specific GMO has been performed.

Redundancy

Biotechnology, like almost all other systems, always operates within a certain level of uncertainty. Accordingly, the principle of redundancy should be employed to reduce the probability of escape of transgenic aquatic organisms by simultaneously employing a number of containment methods/barriers. These methods, each with its own strengths and weaknesses, should be used so that the failure of one under certain conditions will be balanced by the strengths of another. This may involve the use of a number of containment strategies. The Committee on the Biological Confinement of Genetically Engineered Organisms of National Research Council in the USA (NRC 2004) recommends the simultaneous use of multiple containment systems for GM fish. This may include, for example, employing distinct physical containment methods or combining containment methods such as physical and geographic containment. Alternatively, similar barriers can be used in series providing that possible failures would be caused by separate events and the failure of one will not trigger the failure of another. Combining containment methods increases the effectiveness of containment. In a GM aquatic organism facility three to five methods/barriers are generally used and the containment measures adopted depend on the risk assessment of the particular GM aquatic organism in the receiving environment (Kapuscinski 2001)

III. Risk management: Post approval monitoring

One of the major concerns related to R&D projects with GM aquatic organisms is the potential that the GM organism might escape into the environment and trigger a series of irreversible ecological effects (Kapuscinski 2007). The close monitoring and maintenance of the implemented containment measures should therefore be an integral part of the post approval risk management plan. In addition, the immediate outside environment should be monitored for possible GM escapees and possible effects those may have on the environment. An effective monitoring and early detection system could identify potential problems with the containment strategies and help initiate timely remedial actions. An ideal monitoring system should have defined monitoring end points, defined parameters and measureable variables and a sampling scheme, which would allow for statistical analysis. Appropriate sampling methods and analytical techniques must be used to help ensure interpretable data is generated (Senanan et al. 2007).

However, as opposed to confined or unconfined commercial production, the focus during a R&D project in a contained use facility should fall on preventative approaches such as highly effective and redundant containment strategies (i.e. the focus is on containment to manage risk). A full scale post approval monitoring plan is therefore likely to only be applicable for commercial production systems and not for contained R&D facilities for GM aquatic organisms.

IV. Risk communication

In order to promote a clear understanding of the different aspects of risk, including risk assessment and risk management, as well as to provide a better understanding of the particular positions of interested parties, a pathway of risk communication must be established. This will provide information about risk to aid decision-making processes by minimising conflicts and to communicate substantiated perceptions and positions. Parties who could give input could include experts and the public at large as well as other interested and affected parties, as per standard operational procedures of the GMO Act.

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APPLICATION TO REGISTER A FACILITY FOR RESEARCH AND DEVELOPMENT ACTIVITIES INVOLVING GENETICALLY MODIFIED AQUATIC ORGANISMS

This application form should be completed in close consultation with the document "Risk analysis of contained use research and development activities with genetically modified aquatic organisms in South Africa".

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SECTION 1: FACILITY DETAILS

1.1. Details of organisation where the work is to be carried out

University, institute or company etc. carrying out the work

Name of Organisation	
Department	
Physical address	
Postal address	

1.2. Details of person responsible for the work and the facility

Person authorised by university, institute or company as the person managerially responsible for all activities involving genetic modification to be undertaken at the facility. This will often be a Head of a Section, Research Manager, or similar. They are not necessarily involved in genetic modification themselves.

Title, name & surname	
Position	
E-mail address	
Telephone nr.	
Mobile nr.	
Facsimile nr.	
Professional qualification and other relevant training & experience	

1.3. Overview of the proposed activities and the facility

Applying to register (indicate with a X)	- a R&D laboratory facility		OR - a R&D aquaculture facility	
Project title				
GMO(s) to be worked with (common and scientific name(s))				
Proposed GM gene(s)/trait(s) (incl. selectable marker genes)				
Physical address				
Centre coordinates of the proposed facility	S dd^{0} mm.mmm E dd^{0} mm.mmm			
Total surface area of the proposed facility				
Average amount/volume of waste produced per day				
Maximum total contained volume and average batch and/or continuous effluent volume per day				

1.4. Physical location and properties of the facility

Where appropriate, clearly cross-reference the information required in this section to that given in section 1.5.

1.4.1. Describe the site selection process including the reasons, opportunities and constraints that were evaluated in the process.

1.4.2. Describe the current use of the land in a 50m radius of the proposed site.

1.4.3. Provide a topographic map or orthophoto map showing the exact location of the proposed facility in the surrounding area to a minimum radius of 200m(add as **Annexure 1** to this document).

In this map clearly indicate waterways (including streams, ponds, drainage systems such as storm water drains etc.) on the land adjacent to the proposed site (within a 50m or 100m radius for laboratories or growth facilities respectively, unless otherwise requested).

1.4.4. Provide labelled scale diagrams of the proposed facility in two dimensions, including both a site plan and a cross sectional view (add as **Annexure 2** to this document).

In these diagrams clearly indicate all equipment and relevant infrastructure including water supply, effluent water drainage systems (including sewerage). Also indicate the location of work areas where activities with GMOs may take place and the location of airlocks and/or anterooms, filtration, treatment or any other containment systems (as appropriate to the containment level of the facility).

1.5. Risk mitigation features of the facility

For each section specify in the space provided what plans/systems/infrastructures are in place in the facility in compliance of these guidelines. If such plans are not applicable to your facility then the reasons for this must be clearly stated.

Facility features

1.5.1. Indicate risks of escape associated with flooding and facility design features that mitigate these risks. Please take the following into account:

The facility must be designed to prevent the entry of surface run-off water and surface run-off must be diverted around the facility. If the facility is located in an area that is prone to flooding or storm surges, approval of the application must include an assessment of the risks of GMOs escaping during such an event. Such an application must include details on the likelihood and magnitude of the storm surges; facility design features and risk management procedures that ensure that no GMOs are released into the environment.

Risk management procedures might include, for example, removal or destruction of GMOs, and decontamination of all water in the primary containers, secondary containers and effluent treatment system, well before inundation occurs. The scale of the activity conducted in the facility must be small enough to allow time for these procedures to be completed.

For the purposes of these guidelines, flooding includes:

- mainstream flooding (an event where water from a river, lake, estuary, coastal waters or any other water body overflows the natural or artificial banks of the principal watercourses in a catchment);
- flash flooding (flooding that occurs within six hours of the rain which causes the flooding); and
- storm water flooding (local runoff exceeding the capacity of an urban storm water drainage system).
- a storm surge or rise in coastal water levels caused by a storm, cyclone and wind driving water shore-wards.
- tidal waves and tsunamis.

The determination of whether the location of the facility is prone to flooding or storm surges depends on the expected frequency of these events. The facility will be regarded as being prone to flooding if the floor of the facility would be below the 100 year flood level or storm surge event (this equates to a 1 in 100 year flood level or an Annual Exceedance Probability (AEP) of 1%). If it is not possible to obtain the 100 year flood level from the relevant local authority, then the highest 100 year flood level or Defined Flood Level used by that authority will be taken to be the level for determining if the location is prone to flooding or storm surges.

For facilities prone to flooding or storm surges, details about the flooding should include the likely effective warning time that the facility would have prior to inundation and possible hazards that might prevent the implementation of any risk management procedures (such as staff being unable to reach the facility prior to inundation). Applicants should expect the Registrar to seek further details during the assessment of the application.

1.5.2. The facility must be an enclosable space, i.e. physically contained. Depending on the facility category it may be contained within walls, doors, windows, floors and ceilings or fencing, netting and gates. If deemed necessary doors and windows must be lockable. Provide details on the physical containment of the facility to maintain the physical conditions required for containment.

1.5.3. The floors of the facility, including any bunding, must be made of a durable material that is impervious to water and any joins between the floor and the bunding must be sealed. Describe how floors and any bunding have been constructed.

1.5.4. The facility must have a system for the decontamination of liquid effluent and waste that contains, or is suspected of containing, aquatic GMOs or genetically modified (GM) pathogens. Such a system is likely to consist of a number of redundant features to adequately decontaminate liquid effluent and waste. This effluent includes that from the wash-up of equipment such as primary and secondary containers, filters, nets, and any other equipment that does or may contain aquatic GMOs or GM pathogens. If the facility has floor drainage exits, all effluent from these drains must be filtered and/or decontaminated by heat or chemical treatment, before being discharged.

NOTE: The facility may be designed so that the effluent from drainage exits and other liquid effluent from the facility is filtered through or decontaminated by the same system.

Discharge into municipal sewers of effluent that has not been filtered or decontaminated is not an adequate barrier to the unintended escape of aquatic GMOs in most cases because sewers may bypass to storm water systems during high-runoff events, and must not occur in any circumstance for species that might survive passage through the sewer and treatment plants.

Describe systems for the decontamination of liquid effluent.

1.5.5. Indicate whether open spaces between and under benches, cabinets and equipment are accessible for cleaning.

1.5.6. Where any device or system will be connected directly or indirectly to the water supplied to the facility that may allow GMOs to escape into the environment via the water supply to the facility water service, then backflow prevention measures must be implemented.

The water supply to an aquatic facility may come from more than one source. There may be, for example, a dedicated supply for the aquarium systems and a municipal, potable, supply for wash-up and use by facility staff.

If the water supply is a dedicated supply, such as a storage tank dedicated for use inside the facility, then back-siphonage is unlikely and would not need a backflow prevention device. However, if back-pressure from any pumps attached to the water supply might force water back into the tank and the pressure is sufficient to cause overflow from the tank, backflow protection would be necessary.

Give details with regards to the water supply to the facility and indicate measures that will prevent the escape of GMOs into the environment through the water supply, for example the use of backflow prevention measures.

1.5.7. If the work in the facility will involve GM pathogens, or there will be hand contact with GMOs that could persist on the hands after exit from the facility, the facility must contain either a wash basin fitted with tap(s) of the hands-free operation type or some other means of decontaminating hands.

NOTE: Alternatives to wash basins, such as dispensers filled with disinfectant solutions, are considered suitable, provided the dispensers can be operated without using the hands.

Describe measures to decontaminate hands. If necessary refer to the scale diagram of the facility.

1.5.8. If the work in the facility will involve GM pathogens, or there will be contact with any GMOs that could persist on clothing after exit from the facility, then designated storage or hanging provisions for protective clothing must be available in the facility.

Indicate the need for storage or hanging provisions for protective clothing and, if these are necessary, indicate where suitable provisions are available within the facility.

Containment equipment requirements

1.5.9. The facility must be fitted with sufficient primary containers, such as tanks, to contain the aquatic organisms to be kept in the facility. If there is any likelihood of the formation of aerosols containing GM pathogens or viable GMOs (such as the larvae of bivalves and some crustaceans, or viable aquatic plant material) then the aerosols must be prevented from escaping the facility by covering the primary containers or filtering any exhaust air.

Discuss how the facilities primary containers are adequate to contain the aquatic organisms. In addition consider the escape of viable GMOs via aerosols and how these are prevented.

1.5.10. Secondary containment must be provided to retain and decontaminate any spillage of water from:

- breakage of, leakage from, or overflow from, primary container(s); or
- overflow or leakage from aquarium water circulation systems that could contain GMOs.

The total volume to be retained must be calculated to include:

- the volume of a percentage of the primary containers;
- an estimate of the total volume of any potential overflow or leakage from aquarium water circulation systems that could contain GMOs;
- the volume of any disinfectant that might be used; and
- additional capacity to prevent any expected general fluid movement from splashing over the top of the secondary containment.

The percentage of the primary container volume is determined as follows:

- where the number of primary containers is ten or less, the volume of the largest container;
- where the number of primary containers is greater than ten, the total volume of 10% of the containers using the volume of the largest containers. For example for 28 containers, the largest 3 containers must be used to calculate the amount.

In facilities where the total volume of water to be retained is sufficiently small relative to the floor area of the facility such that water cannot flow out of the facility, there may be no need for secondary containment.

The secondary containment could consist of bunding or some other containment such as holding trays under individual tanks or racks of tanks. If the facility has an effluent decontamination system for retaining drainage from the facility floors, and the system is large enough to retain and treat any overflow or leakage that drains onto the floor, then the effluent decontamination system may be used as the secondary containment.

With regards to accident prevention and emergency response plans the following information must also be provided.

- Information on the source of hazards and conditions under which accidents occur.
- Information on the preventative measures applied such as safety equipment, alarm systems, containment methods and procedures and available resources.
- A description of information given to workers.
- A summary of emergency plan/s prepared prior to the commencement of the activity.
- Policy guidelines for ancillary and maintenance staff, contractors and visitors.
- Information on the maintenance and test procedures of safety equipment. This Must include equipment such as ventilation systems, effluent filter systems, high efficacy particulate air filters (HEPA), decontamination systems and other safety equipment.
- Guidelines for ancillary and maintenance staff, contractors and visitors.
- Information on health surveillance which should, where appropriate, include Screening procedures including the immune status of the individual, sickness investigation, issue if medical contact cards, immunisation procedures, maintenance of baseline serum samples for staff.
- Information on the duties of the biological safety officer.

Discuss the need for secondary containment measures within the facility and accident prevention and emergency response plans.

Waste management

1.5.11. Please provide the following information with regards to waste management.
Provide details of waste treatment including types of waste, quantities, potential hazards and levels of live genetically modified micro-organisms in the waste.

1.5.12. Information on the waste management techniques used, including recovery of liquid or solid waste and the inactivation techniques used.

1.5.13. Information on the ultimate form and destination of inactivated waste.

SECTION 2: BIOSAFETY RISK ASSESSMENT

- Each project must be accompanied by a risk assessment.
- Below is a proposed risk assessment framework. Further sections, as appropriate to your application may be added, however for guidance and regulations please refer to the requirements of the Department of Agriculture, Forestry and Fisheries (available at www.daff.gov.za) and the provisions of the Occupational Health and Safety Act, 1993 and applicable regulations required by the Department of Labour.

2.1. Title of the project

2.2. Purpose of the activity to be undertaken and intended use of the GMO

Including new or changed use compared to the unmodified organism.

2.3. Characteristics of the unmodified, RECIPIENT ORGANISM

- Common and taxonomic names (genus, species, subspecies and strain as appropriate) of the recipient organism, i.e. the aquatic organism that will be genetically modified.

- List the centre(s) of origin and, if known, the centres of genetic diversity of the organism.

Give an overview of the biology of the aquatic organism, including:

- Describe the reproductive cycle of the organism, including details on all the stages of its life cycle.
- The organism's natural habitat and geographical distribution.
- The characteristics of the organism's habitat and environmental tolerances of he organism with the view to identify habitats where it may persist or proliferate, i.e. predict possible habitats.
- Describe any possible significant involvements of the organism in ecological processes.
- Describe the interaction of the organism with other organisms in the environment and its effect on those organisms, including its likely competitive or symbiotic properties.
- The host range if the organism is a parasite or pathogen.

- With reference to the recipient organism's different life stages, what are the possible pathways through which the organism or its gametes could escape from the facility? Including for example eggs and sperm, which are transmitted into the water column or larvae that have a planktonic or swimming form.

- List and discuss any other potentially significant physiological and genetic traits, relevant to biosafety of the recipient organism.

- Discuss the nature of any possible pathogenicity, virulence, infectivity or toxicity of the organism and / or its potential to act as a vector of disease transmission.

- What is the geographical source/point of acquisition of the recipient organism?

- Is the same species or closely related species found in the likely receiving environment in the event of an accidental bridge in containment?

- If the organism has been imported specifically for this project, have all the other relevant authorities approved the importation of these organisms and were these organisms certified as disease free?

- Is the wild type species of the aquatic organism an undesirable species or on a list of undesirable species?

- Are living specimens of the recipient organism currently being imported into South Africa for any other reasons?

2.4. Characteristics of the DONOR ORGANISM(S) and the TRANSGENIC ELEMENTS

- Common and/or taxonomic names (genus, species, subspecies and strain as appropriate) of the donor organism(s), i.e. the organisms from which the various transgenic elements are obtained, and the corresponding transgenic element(s) (designation and function).

Donor organism	Transgenic element

- Degree of relatedness between the recipient and any of the donor organisms.

- List and discuss any other potentially significant characteristics, relevant to biosafety of the donor organism(s) and the donated transgenic elements.

2.5. Characteristics of the intended GENETICALLY MODIFIED ORGANISM (GMO)

- History of any (national and/or international) prior genetic modifications to the recipient organism, including stage of development.

- Comment on the general stability of the genetic traits of the organism.

- Give the base sequence(s), frequency of mobilization and specificity of the organism's endogenous vectors if present and available.

- Give a description of the genetic modification, including the technique used or proposed to be used to introduce a vector or insert into the organism.

- What is the identity, nature, source/origin, host range and genetic transfer capability of the genetic vector introduced into the organism?

- Give the function (associated trait) of the genetic modification(s) and/or of all new, intentionally introduced gene sequence(s), including any marker genes that may have been used?

- Discuss the likely structure, amount and possible impact of any additional sequences, e.g. Vector or donor organism sequences, remaining in the final construction of the genetically modified organism.

- Give the function/activity of all potentially expressed transgenes/proteins.

- What are the likely/preferred rate, level and specificity of expression of the respective new genes in the organism? Also indicate the method(s) and the sensitivity of measurements to determine/confirm these.

- Give information on the presence of any genes in the organism, which confers resistance to antibiotics or any other selection agent, including both endogenous and transgenes.

- What is the estimated number of GM individuals that will be used in the facility? Include numbers of GMOs that will be used per experiment and possible number of GM variants, i.e. transformed with different constructs or combinations thereof.

- If the organisms are to be grown into adult life stages please indicate how the GMOs will be identified. This could include the use of labels on containers or marking the organisms.

2.6. Characteristics of the RECEIVING ENVIRONMENT and other environmental considerations

The receiving environment for contained use R&D facilities for GMOs includes both the immediate environment outside the facility and more distant environments, which could be accessible to living GMOs (LMOs) through plausible pathways of persistence and dispersal, which will be exposed to the GMO if there is an accidental bridge in containment.

- Give a general overview of the geographical, climatic and ecological characteristics, including relevant information on biological diversity and possible centres of origin or diversity for other organisms, of the likely receiving environment.

- Discuss the factors affecting the survival, multiplication and dissemination of the modified organism in the environment. Refer to the information already supplied for the recipient organism and focus on possible changes due to the genetic modifications. Again, reference must be made to all stages of reproduction, specifically their mobility and dispersal mechanisms that could increase the likelihood of the GMO escaping the facility.

- Integrate the information supplied above by discussing the possible survival, persistence, dispersal and/or proliferation of the GMO in case of an accidental bridge in containment. Refer specifically to suitable habitats in the immediate environment and/or other plausible persistence pathways through which the GMO could be dispersed to more distant suitable habitats.

- If any possible persistence pathways exist for the GMO, identify the ecosystems to which the modified organism could disseminate.

- Determine the anticipated mechanisms and results/impacts of interactions between the GMO and other organisms in exposed ecosystems. Describe the known or predicted effects of the organism on other plants and animals, including pathogenicity, infectivity, toxicity, virulence, vector or pathogen allergenicity, colonisation, predation, parasitism, symbiosis and competition.

- Discuss the availability and expected effectively of methods for decontamination/removing the GMO from the receiving environment, in case of accidental exposure.

- Identify available phenotypic and/or molecular techniques for the detection, identification and monitoring of the modified organism in the environment.

- Identify available methods and techniques for detecting possible transfer of the transgenic material to other organisms.

2.7. Human health considerations

- Give the toxic or allergenic effects of viable and non-viable organisms and/or their metabolic products (if any)?

- Provide all possible health hazards associated with the GMO.

- Compare the GMO to the donor and recipient organisms in terms of their respective, its potential pathogenicity. Include the capacity of the organisms to colonise humans, requirements for immunocompetency, the diseases caused, invasiveness, virulence, communicability, infective dosage, host range, possible alterations, biological stability, survival outside of a human host, vectors or means of dissemination and possible resistance mechanisms.

- Discuss the availability of appropriate therapies.

- What are the implications of the proposed activity with regard to the health and safety of the workers, cleaning personnel and any other person that will be directly or indirectly involved in the activity? Please take into consideration the provisions of the Occupational Health and Safety Act, 1993 (Act No. 181 of 1993) and accompanied regulations.

- Indicate the proposed health and safety measures that would be applied to safeguard employees during the proposed activities.

2.8. Monitoring and accident

- Indicate the methods and plans for monitoring the GMO.

- Indicate any emergency procedures that will be applied in the event of an accident.

2.9. Biosafety risk assessment

Using the above information, perform a comprehensive biosafety risk assessment to estimate the overall risk, associated with the proposed activities with the GMO(s), to the environment and human health. For each of the identified hazards, to the environment or human health, give an overall measure of the level of risk. The use of a risk assessment matrix (see guidance document "Risk analysis of contained use activities with genetically modified aquatic organisms in South Africa") will assist with estimating the level of risk by considering the likelihood and consequence of a harm.

In a contained use facility appropriate risk mitigation measures, primarily containment measures, can be employed to reduce the likelihood of a harm occurring and thereby reducing the overall level of risk to acceptable levels.

Environmental risk estimate.

- Identify all potential hazards and the conditions (i.e. plausible pathways) under which these could result in harm to the environment if the GMO is unintentionally released into the environment.
- Consider the likelihood and consequence of all potential harms and before and after taking into consideration all the incorporated risk management strategies/containment measures and give the risk estimate for each, i.e. negligible to high.
- If no environmental hazards are foreseen the reasons for making this assumption should be supported with relevant information.

- Are the risk mitigation features of the facility / activities described above adequate to reduce the identified risks to acceptable levels?

- What do you consider the overall risk of the proposed activities to be to the environment i.e. have all identified risks been managed so that activities take place within an acceptable level of risk to the environment?

Human health risk estimate

- Identify all potential hazards and the conditions (i.e. plausible pathways) under which these could result in harm to the health of the general human population if the GMO is unintentionally released into the environment.
- Consider the likelihood (including all the incorporated risk management strategies / containment measures) and consequence of all potential harms and give the risk estimate for each, i.e. negligible to high.
- If no environmental hazards are foreseen the reasons for making this assumption should be supported with relevant information.

- Are the risk mitigation features of the facility/activities described above adequate to reduce the identified risks to acceptable levels?

- What do you consider the overall risk of the proposed activities to the health of the general human population, i.e. have all identified risks been managed so that activities take place within an acceptable level of risk to human health?

AFFIDAVIT / VERKLARING / STATEMENT

Moet ingevul word in die teenwoordigheid van „n Kommissaris van Ede. Must be completed in the presence of a Commissioner of Oaths.

Ek/I _____

ID-Nommer/Number _____

Woonadres/ Residing address _____

Werkadres/working address _____

Tel(w)_____ (cell)_____(h)_____

Verklaar onder eed in Afrikaans / bevestig in Afrikaans:
Ek is vertroud met die inhoud van bostaande verklaring en begryp dit. Ek het geen beswaar/het beswaar teen die aflê van die voorgeskrewe eed. Ek beskou die voorgeskrewe eed/bevestiging as bindend vir my gewete.

Declare under oath in English / confirm in English:
I am familiar with, and understand the contents of this declaration. I have no objection/have objection to taking the prescribed oath. I consider the prescribed oath as binding to my conscience.

Plek/Place _____ Datum/Date _____ Tyd/Time _____

Handtekening/Signature _____

Kommisaris van Ede/Commissioner of Oaths

Ek sertifiseer dat bostaande verklaring deur my afgeneem is en dat die verklaarder erken dat hy/sy vertroud is met die inhoud van hierdie verklaring and dit begryp. Hierdie verklaring is voor my beëdig en verklaarder se handtekening/merk/duimafrduk is in my teenwoordigheid daarop aangebring.

I certify that the above statement was taken from me and that the deponent has acknowledge that he/she knows and understands the contents of the statement. The statement was sworn to/affirmed before me and deponent"s signature/mark/thumb print was placed thereon in my presence.

Te/At _____ op/on _____ om/at _____

Handtekening/Signature _____

(Inligting i.v.m. fisiese en posadres moet verskaf word, bv. stempel van die polisiestasie. Details to be provided on physical and postal address, e.g. stamp of police station)

_____

Magsnommer /Rang/Naam (in drukskrif) Force number/Rank/Name (in print)

SECTION 3: FACILITY MANAGEMENT AND CONDITIONS OF REGISTRATION

All authorised persons must be familiar with the Facility Management requirements as set out below.

3.1. Facility access

Access to the facility must be restricted to authorised persons.

NOTE: Access can be restricted by means such as: keys, key cards or combination locks for entry to the facility; or controlled access to the building where the registered facility is only a part of a larger building.

3.2. Facility personnel training and record keeping

All facility personnel must be trained in the use of equipment present in the facility and also in the procedures to be used in the facility. Records of this training must be kept and made available to the Registrar if requested.

NOTE: The required training should include transport, disposal, identification of hazards associated with the GMO and emergency procedures, registration requirements and contained use conditions.

3.3. Facility inspections

The facility must be inspected at least once every 12 months. The inspection report must detail the extent of compliance with the conditions of registration and a copy of the last 5 year"s inspection reports must be provided to the Registrar if requested.

NOTE: The permit holder can arrange for any competent person to inspect the facility to assess compliance with these conditions of contained activities with an aquatic GMO.

3.4. Personnel protective clothing and equipment

The following personal protective clothing must be worn by personnel performing procedures in the facility:

- laboratory coat or gown, or equivalent, to protect the arms and front part of the body from spills or any other source of contamination (when performing procedures that involve GM pathogens or there is contact with GMOs that could persist on the clothing after exit from the facility); and

- gloves (when performing procedures that might lead to contamination of the hands, if working with GM pathogens that are hazardous to humans).

If the work in the facility involves GM pathogens, or there is contact with GMOs that could persist on the clothing after exit from the facility, then laboratory coats, gowns or equivalent, must be removed before leaving the facility and stored in designated storage or hanging provisions. This condition does not apply if moving directly to another containment facility, registered to the same containment level or higher, that is directly connected to the facility.

3.5. Work practices and personnel decontamination

Facility doors must remain closed while work is being undertaken in the facility and must be locked when the facility is unattended.

- The facility windows must be closed and locked while GMOs are in the facility.
- Workbenches, surfaces and equipment must be decontaminated after completion of procedures involving GM pathogens or GMOs that could persist on facility surfaces.
- All work surfaces and equipment where maintenance is to be carried out must be decontaminated prior to maintenance taking place if procedures involving GM pathogens or GMOs that could persist on facility surfaces have been conducted there.
- Persons who have been performing procedures in the facility that involve GM pathogens, or where there has been hand contact with GMOs that could persist on the hands after exit from the facility, must decontaminate their hands before leaving the work area. This can be achieved by washing, or use of appropriate chemical decontaminant.

NOTE: Alternatives to wash basins, such as dispensers filled with disinfectant solutions, are suitable, provided the dispensers can be operated without using the hands.

3.6. Decontamination of GMOs and equipment

Decontamination of GMOs or items or material contaminated with GMOs must be performed as follows:

- GMOs and aquatic organisms containing GMOs must be rendered non-viable prior to disposal.

- All liquid effluent that contains, or is suspected of containing, aquatic GMOs or GM pathogens, must be decontaminated before discharge to prevent the escape of viable GMOs. The decontamination method must be effective against the GMOs that may be contained in the effluent.

- Solid wastes containing GMOs must be decontaminated prior to disposal.

- Equipment must be decontaminated prior to use in another location.

- Protective clothing contaminated with GM pathogens or with GMOs that can persist on the clothing must be taken off as soon as practicable and decontaminated prior to reuse. Protective clothing that has not been contaminated with GM pathogens may be washed using normal laundry methods.

Except for liquid effluent, decontamination can take place in the work area of the facility, or at another location providing the organisms or waste are transported to the decontamination site in accordance with any transport guidelines and other relevant guidelines.

NOTE. Effluent includes that from the wash-up of equipment such as primary and secondary containers, filters, nets, and any other equipment.

3.7. Appropriate decontamination methods

Decontamination can be effected by physical containment methods such as pressure steam sterilisation (autoclaving) or other heat treatment; chemical treatment; incineration; or by any other approved method.

- Any heat treatment must be performed using a combination of temperature and time that has been validated as effective against the organisms being rendered non-viable.
- Chemical disinfectant treatment must be effective against the organisms being rendered non-viable.
- Incineration must be performed in a high temperature, high efficiency incineration facility that has been approved by the relevant government authority in the jurisdiction where the incinerator is located.

Where use of a pressure steam steriliser (autoclave) is required for sterilisation purposes:

- Loads must be packed and loaded to allow for the penetration of steam into the material being sterilised.
- The coldest part of the load must be exposed to a minimum temperature of 121°C for at least 15 minutes.
- Measures must be taken to ensure that loads that have been processed can be differentiated from loads that have not (e.g. by use of autoclave tape).
- The efficacy of the sterilisation treatment must be validated at least monthly by the use of:
- thermocouples or resistance thermometers, to ensure that the sterilisation temperature has been achieved; or
- chemical indicators which progressively change colour with the time exposed at the specified temperature; or biological indicators such as spore strips; or enzyme indicators.

Calibration of the autoclave thermometer and timers, and pressure testing of the vessel, must be performed annually by a competent person. The results of the autoclave tests, including evidence of the calibration of the equipment used, must be kept for the previous 5 years and made available to the Registrar, if requested.

If an autoclave is found to be defective and the defect has not been corrected, the autoclave must be clearly marked to show that it is defective and must not be used for decontaminating organisms, waste or equipment associated with activities with GMOs until the defect has been corrected.

3.8. Removal of organisms from the facility that are not GMOs

Organisms that are not GMOs must not be removed from the facility while an activity with a GMO is occurring in a facility unless:

- procedures are implemented to ensure that activities with GMOs do not mix with or contaminate work with any other organisms that are not part of the activity;
- the above procedures are documented; and
- all primary containers and transport containers are decontaminated prior to removal from the facility.

If mixing or cross-contamination of any other work by GMOs occurs, or is suspected to have occurred, then the other work must be handled and disposed of in accordance with the conditions of activities with an aquatic GMO, as if it were an activity with a GMO.

NOTE: Means of preventing cross-contamination of other work by GMO activities could include physical separation of the work, or separation by working at different times and ensuring any contaminated surfaces are decontaminated prior to commencing different work.

3.9. Removal of GMOs from the facility

Viable aquatic GMOs must not be removed from the facility unless:

- they are to be transported to a registered aquatic organism containment facility registered by the Registrar to a containment level equal to or higher than the facility from which it is being removed;
- they are to be transported to another location for storage;
- they are rendered non-viable prior to disposal; or
- written permission has been given by the Registrar for transport to another destination.

3.10. Transport of GMOs

All GMOs, and material contaminated with GMOs, being transported out of the facility must be transported in accordance with any transport guidelines and other relevant guidelines.

Transport of GMOs between the registered facility and any storage unit must be in accordance with any transport guidelines and other relevant guidelines.

3.11. Containment of GMOs outside the registered facility

GMOs or organisms containing or infected with GMOs being stored outside the registered facility must be double contained. The primary container must be sealed and leak proof. The primary container must be clearly labeled so it can be identified and must be stored in an unbreakable secondary container. In the case of a small storage unit such as a fridge, freezer or liquid nitrogen container, the secondary container may be the storage unit.

All primary containers must be labeled to enable identification of the organism being contained

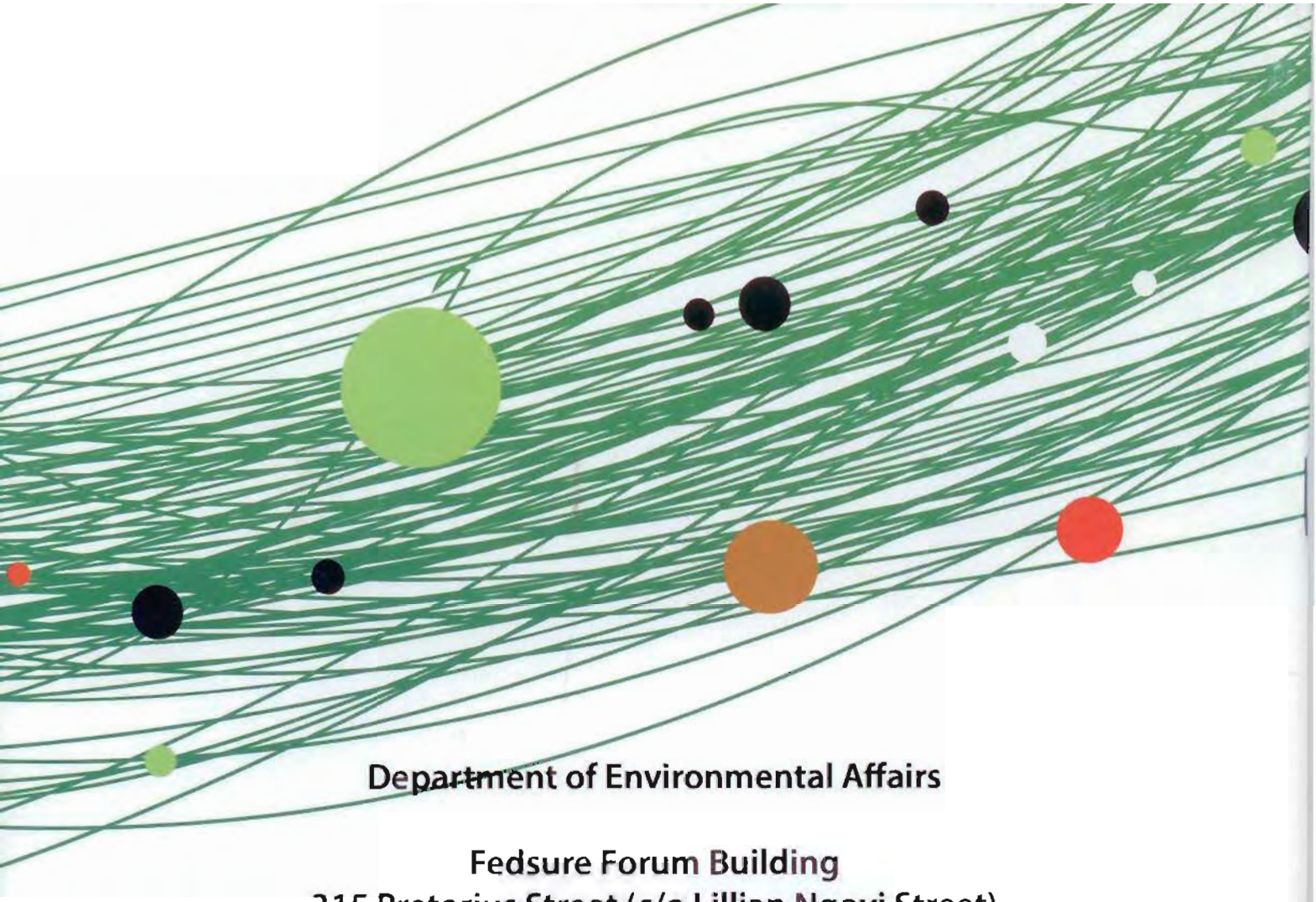
NOTE: 'Unbreakable' in this context means the container will not break open and spill any of its contents if dropped on the floor.

3.12. Spills outside the registered facility

Any spills of GMOs that occur outside the registered facility must be reported to the Registrar as soon as practicable. The spilt material and any contaminated surfaces must be decontaminated.

3.13. Unintentional breech in containment

Any unintentional release or suspected unintentional release of GMOs from the facility must be reported to the Registrar as soon as practicable.



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