Annual Review of Risk Assessment Made Under:
Genetically Modified Organisms (Contained Use) Regulations 2014

Department: Nuffield Division of Clinical Laboratory Sciences
Radcliffe Department of Medicine

Supervisor: Prof Stephen Hyde

Ref No: CBGM15

Title: Transgenic Animals

The Risk Assessment has been reviewed: ..... YES
Key aspects: identification of any potentially harmful effects, characteristics of the proposed activity, the severity of any potentially harmful effects, the likelihood of them occurring and disposal of waste and effluent.

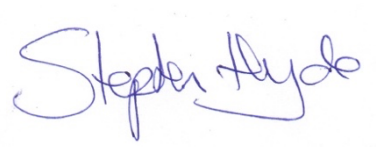
Appropriate containment measures have been confirmed:..... YES
Complete attached containment levels/measures table

Original containment level and risk classification remain valid:. YES

Classification and assignment of final control measures:
Containment Level: CL1
Risk Classification: 1

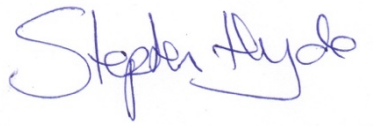
What has changed? Updated list of users

Reviewed By:
Date (YYYY-MM-DD):



Prof Stephen Hyde
2025-10-16

Approved By Genetic Modification Safety Committee
Agreed By One-Of DSO/BSO/HoD:
Date (YYYY-MM-DD):



Prof Stephen Hyde – NDCLS BSO
2025-10-29

Approved by Head of Department
Date (YYYY-MM-DD):

Prof Deborah Gill – NDCLS HoD
2025-10-29

Next Review Due:
Before end 2026

Updated List Of Associated Transgenic Sequences:

Similar risks are associated with transgenic animals harbouring a range of other non-harmful transgenic modifications including:

Common Reporter Genes:
EGFP and similar proteins

Bacterial Proteins
Staphylococcus aureus Cas9 (saCas9) and similar proteins along with associated gRNA and similar sequences.
Cre recombinase and similar proteins
Hybrid tetracycline repressor/transcriptional activation/control and similar proteins

Mammalian ion channels/transporters proteins:
Cystic fibrosis transmembrane conductance regulator (CFTR),
ATP-Binding Cassette, Sub-family A, Member 3 (ABCA3)

Mammalian secreted proteins:
alpha-1 anti trypsin (SERPINA1),
surfactant protein A to D (SFTPA-SFTPD)

Risk Assessment Users & Supervisor During Year To Review Date
Stephen Hyde
Emily Castells (Stephen Hyde)
Marina Cerezuela (Stephen Hyde)
Hamid Dolatshad (Stephen Hyde)
Kamran Miah (Stephen Hyde)
Eoin Mac Reamoinn (Stephen Hyde)
Aimee Ruffle (Stephen Hyde)
Gavin Turnbull (Stephen Hyde)
Stephanie Jones (Stephen Hyde)
Shahzaib Tariq (Stephen Hyde)

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Complete attached containment levels/measures table

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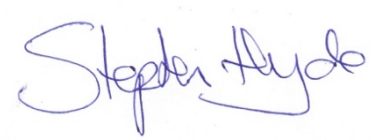
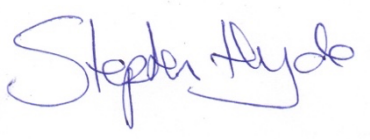
Classification and assignment of final control measures:
Containment Level: CL1
Risk Classification: 1

Reviewed By:
Date (YYYY-MM-DD):



Prof Stephen Hyde
2024-08-16

Approved By Genetic Modification Safety Committee
Agreed By One-Of DSO/BSO/HoD:
Date (YYYY-MM-DD):



Prof Stephen Hyde – NDCLS BSO
2024-10-02

Approved by Head of Department
Date (YYYY-MM-DD):

Prof Deborah Gill – NDCLS HoD
2024-10-02

Next Review Due:
Before end 2025

Updated List Of Associated Transgenic Sequences:

Similar risks are associated with transgenic animals harbouring a range of other non-harmful transgenic modifications including:

Common Reporter Genes:
EGFP and similar proteins

Bacterial Proteins
Staphylococcus aureus Cas9 (saCas9) and similar proteins along with associated gRNA and similar sequences.
Cre recombinase and similar proteins
Hybrid tetracycline repressor/transcriptional activation/control and similar proteins

Mammalian ion channels/transporters proteins:
Cystic fibrosis transmembrane conductance regulator (CFTR),
ATP-Binding Cassette, Sub-family A, Member 3 (ABCA3)

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Table 1a Containment measures applicable to contained use involving micro-organisms in laboratories

Containment Measures		**Containment Levels**			
		CL1	CL2	CL3	CL4
Facilities					
1	Laboratory suite: isolation[1]	not required	not required	required	required
2	Laboratory: sealable for fumigation	not required	not required	required	required
Equipment					
3	Surfaces impervious to water, resistant to acids, alkalis, solvents, disinfectants and decontamination agents and easy to clean	required for any bench	required for any bench	required for any bench and floor	required for any bench, floor, ceilings and walls
4	Entry to laboratory via airlock[2]	not required	not required	required where and to extent the risk assessment shows it is required	required
5	Negative pressure relative to the pressure of the immediate surroundings	not required	not required	required except for activities where transmission does not occur by the airborne route	required
6	Extract and input air from the laboratory must be HEPA filtered	not required	not required	HEPA filters required for extract air except for activities where transmission does not occur by the airborne route	HEPA filters required for input and extract air[3]
7	Microbiological safety cabinet/ enclosure	not required	required where and to extent the risk assessment shows it is required	all procedures with infective materials required to be contained within a cabinet/ enclosure	required, and all procedures with infective materials required to be contained within a cabinet/ enclosure
8	Autoclave	required on site	required in the building	required in the laboratory suite[4]	double ended autoclave required in laboratory

Containment Measures		Containment Levels			
		CL1	CL2	CL3	CL4
System Of Work					
9	Access restricted to authorised personnel only	not required	required	required	required (via airlock key procedure)
10	Biohazard sign on door	not required	required	required	required
11	Specific measures to control aerosol dissemination	not required	required so as to minimise	required so as to prevent	required so as to prevent
12	Shower	not required	not required	required where and to extent the risk assessment shows it is required	required
13	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	complete change of clothing and footwear required before entry and exit
14	Gloves	not required	required where and to extent the risk assessment shows they are required	required	required
15	Efficient control of disease vectors (eg rodents and insects) which could disseminate GMMs	required where and to extent the risk assessment shows it is required	required	required	required
Waste					
16	Inactivation of GMMs in effluent from hand- washing sinks and showers and similar effluents	not required	not required	required where and to extent the risk assessment shows it is required	required
17	Inactivation of GMMs in contaminated material and waste	required by validated means where and to extent the risk assessment shows it is required	required by validated means	required by validated means, with waste inactivated within the laboratory suite	required by validated means, with waste inactivated within the laboratory

Containment Measures		Containment Levels			
		CL1	CL2	CL3	CL4
Other Measures					
18	Laboratory to contain its own equipment	not required	not required	required, so far as is reasonably practicable	required
19	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 where and to extent the risk assessment shows it is required	required
20	Safe storage of GMMs	required where and to extent the risk assessment shows it is required	required	required	secure storage required
21	Written records of staff training	not required	required where and to extent the risk assessment shows it is required	required	required

1 “isolation” means, in relation to a laboratory, separation of the laboratory from other areas in the same building, or being in a separate building.

2 Entry must be through an airlock which is a chamber isolated from the laboratory. The clean side of the airlock must be separated from the restricted side by changing or showering facilities and preferably by interlocking doors.

3 Where viruses are not retained by the HEPA filters, extra requirements will be necessary for extract air.

4 Where the autoclave is outside the laboratory in which the contained use is being undertaken, but within the laboratory suite, there must be validated procedures for the safe transfer of material into that autoclave, which provide a level of protection equivalent to that which would be achieved by having an autoclave in that laboratory.

Table 1b Containment measures applicable to contained use involving micro-organisms in plant growth facilities (to be read with Table 1a)

Omitted as not relevant to NDCLS activities

Table 1c Containment measures applicable to contained use involving micro-organisms in animal units (to be read with Table 1a)

Containment Measures		**Containment Levels**				Additional / Modification
		CL1	~~CL2~~	~~CL3~~	~~CL4~~	
Facilities						
1	Isolation of animal unit[1]	required where and to extent the risk assessment shows it is required	~~required~~	~~required~~	~~required~~	modification
2	Animal facilities[2] separated by lockable doors	required where and to extent the risk assessment shows it is required	~~required~~	~~required~~	~~required~~	additional
3	Animal facilities (cages, etc) designed to facilitate decontamination (waterproof and easily washable material)	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~~	~~required~~	additional
4	Floor, walls and ceiling easily washable	required where and to extent the risk assessment shows it is required	~~required for floor~~	~~required for floor and walls~~	~~required for floor, walls and ceiling~~	Modification
5	Appropriate filters on isolators or isolated rooms[3]	not required	~~required where and to extent the risk assessment shows it is required~~	~~required~~	~~required~~	additional
6	Appropriate barriers at the room exit, and at drains or ventilation duct work	required	~~required~~	~~required~~	~~required~~	additional
7	Animals kept in appropriate containment facilities, such as cages, pens or tanks but not isolators	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 where and to extent the risk assessment shows it is required~~	~~required where and to extent the risk assessment shows it is required~~	Additional
8	Animals kept in isolators	not required	~~required where and to extent the risk assessment shows it is required~~	~~required~~	~~required~~	modification

1 "animal unit" means a building, or separate area within a building, containing an animal facility and other areas including changing rooms, showers, autoclaves and food storage areas.

2 "animal facility" means a facility normally used to house stock, breeding or experimental animals or one which is used for the performance of minor surgical procedures on animals.

3 "isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be more appropriate

Risk Assessment made under the Genetically Modified Organisms (Contained Use) Regulations 2000

FOR THE GENERATION, BREEDING, OR USE OF GENETICALLY MODIFIED ANIMALS

Department: Nuffield Department of Clinical Laboratory Sciences **Ref. No:** **CBGM 15**

Supervisor: Dr Stephen Hyde

Title and Outline Description of Project:

Breeding of CFTR knockout mice

Cystic Fibrosis (CF) is a common inherited disease of humans. Mice defective for the murine cystic fibrosis transmembrane conductance regulator (CFTR) gene, generated elsewhere, will be bred for scientific studies aimed at increasing the understanding of the ion transport defects and pathophysiology of CF.

Type of animals involved:

Mice.

Describe how the transgenic animal was/will be produced: ***(eg micro injection, transfection of embryonic stem cells, viral vector).***

Transfection of embryonic stem cells.

If new genetic material is incorporated into the animals, state source of the genetic material:

The neomycin resistance gene from *E.coli* transposon Tn903 was inserted into the murine CFTR locus by gene targeting.

Is the genetic material capable of horizontal transmission other than as a chromosomal element?

No

What effect is the modification likely to have on the transgenic animal? ***(give full details, where known state effect of mutation/damage/over-expression etc of the gene in the donor species, for knock-outs give expected effect of losing the gene, consider also whether any physical or behavioural changes are likely to result).***

The murine CFTR gene was inactivated in embryonic stem cells by targeted insertion of the neomycin resistance gene (Koller et al., 1991 PNAS 88:10730). The disrupted CFTR gene contains an in frame stop mutation in exon 10 designated S489X. This truncated gene product is similar to that seen in several types of human CF mutations. In humans, this type of mutation results in a severe CF phenotype similar to the highly prevalent ΔF508 mutation.

CFTR knockout mice typically have a defect in intestinal physiology, which is expected to result in a failure to thrive, and increased post-natal mortality. The majority of this post-natal mortality occurs within a few days of birth and in the period of around weaning. It is expected that the incidence of post-natal mortality can be reduced by the use of reduced fibre diet and bedding.

CFTR knockout mice are typically smaller than unaffected littermates. CFTR knockout mice do not typically show abnormal behaviour.

If the animal were to escape could it

- have any selective advantage over the wild type population?

No

- result in problems associated with transmission of manipulated genes to other animals?

No

- cause any particular problems/adverse effects to the environment?

No

If the transgenic animal were to bite or scratch someone could the modification lead to any additional risks to humans compared to a bite or scratch from a wild type animal?

(if yes, please describe).

No

The transgenic animals will be held at:

Animal Containment Level A ~~B~~ *(please delete as appropriate)*

Is the transgenic animal going to be infected/inoculated with a genetically modified micro-organism

~~YES~~/NO *(if yes complete also Tables 1a and 1c)*

Give location where animals will be held and what procedures will be undertaken:

The animals will be bred in the designated medical and scientific departments of the University of Oxford Biomedical Services, Oxford.

Does the transgenic animal pose a greater risk of harm to human health and safety than the non-modified equivalent?

~~YES~~/NO

Notes

(1) In order to undertake the work detailed above the requirements of the Animals (Scientific Procedures) Act 1986 must also be met. A Project Licence and Personal Licence will be required. If necessary, investigators should contact Veterinary Services for further details.

(2) The person responsible for the day to day care of the animals in the holding facility must be informed that transgenic animals are being used and relevant health and safety information provided.

(3) All carcasses and tissue waste must be disposed of by incineration as clinical waste. The use of surplus animals for other purposes must be risk assessed. Carcasses, tissues or surplus animals must not be used as feedstuffs for other animals.

Assessed By: Dr Stephen Hyde

Signature: Stephen Hyde Date: 6/10/2004

Risk Assessment approved by Genetic Modification Safety Committee **Yes/No**

Signature: Stephen Hyde Date: 6/10/2004

(Biological Safety Officer)

Permission granted by Head of Department for project to be undertaken **Yes/No**

Signature: Date: 6/10/04

(Head of Department)

Table 1a: Containment Measures for Activities involving GMMs in Laboratories

Where a item is listed as "may be required" this indicates the item to be an option at that particular containment level and its requirement should be determined by the risk assessment for the particular activity concerned. Delete no or yes as indicated by risk assessment.

Containment Measures	Containment Levels			
	1	2	3	4
Isolated laboratory suite	not required	~~not required~~	~~required~~	~~required~~
Laboratory sealable for fumigation	not required	~~not required~~	~~required~~	~~required~~
Surfaces impervious, resistant and easy to clean	required for bench	~~required for bench~~	~~required for bench and floor~~	~~required for bench, floor, ceiling and walls~~
Entry to lab via airlock	not required	~~not required~~	~~may be required no / yes~~	~~required~~
Negative pressure relative to the pressure of the immediate surroundings	not required	~~may be required no / yes~~	~~required~~	~~required~~
HEPA filtered extract and input air	not required	~~not required~~	~~required for extract~~	~~required for input and extract~~
Microbiological safety cabinet/enclosure	not required	~~may be required no / yes~~	~~required~~	~~required (class 3)~~
Autoclave	required on site	~~required in the building~~	~~required in the lab suite~~	~~required in lab (double ended)~~
Access restricted to authorised personnel	not required	~~required~~	~~required~~	~~required~~
Specified measures to control aerosol dissemination	not required	~~required so as to minimise~~	~~required so as to prevent~~	~~required so as to prevent~~
Shower	not required	~~not required~~	~~may be required no / yes~~	~~required~~
Protective clothing	suitable protective clothing required	~~suitable protective clothing required~~	~~suitable protective clothing required~~	~~complete change of clothing and footwear~~
Gloves	not required	~~may be required no / yes~~	~~required~~	~~required~~
Control of disease vectors (eg rodents, insects) which could disseminate GMMs	may be required no / ~~yes~~	~~required~~	~~required~~	~~required~~
Specified disinfection procedures in place	may be required no / ~~yes~~	~~required~~	~~required~~	~~required~~
Inactivation of GMMs in effluent from handwashing sinks, showers etc	not required	~~not required~~	~~may be required no / yes~~	~~required~~
Inactivation of GMMs in contaminated material and waste	required by validated means	~~required by validated means~~	~~required by validated means~~	~~required by validated means~~
Laboratory to contain its own equipment	not required	~~not required~~	~~required~~	~~required~~
An observation window or alternative so that occupants can be seen	may be required no / ~~yes~~	~~may be required no / yes~~	~~required~~	~~required~~
Safe storage of GMMs	may be required no / ~~yes~~	~~required~~	~~required~~	~~secure storage required~~
Written records of staff training	not required	~~may be required no / yes~~	~~required~~	~~required~~
CLASSIFICATION	**CLASS 1**	**~~CLASS 2~~**	**~~CLASS 3~~**	**~~CLASS 4~~**

Table 1c: Containment Measures for Activities involving GMMs in Animal Units - TABLE 1a TO BE COMPLETED WITH THE FOLLOWING ADDITIONS/MODIFICATIONS:

Where a item is listed as "may be required" this indicates the item to be an option at that particular containment level and its requirement should be determined by the risk assessment for the particular activity concerned. Delete no or yes as indicated by risk assessment.

Containment Measures	Containment Levels				Addition/ modification
	1	2	3	4	
Isolation of animal unit (note 1)	may be required ~~no~~ / yes	~~required~~	~~required~~	~~required~~	
Animal facilities (note 2) separated by lockable doors	may be required ~~no~~ / yes	~~required~~	~~required~~	~~required~~	
Animal facilities (cages etc) designed to facilitate decontamination (waterproof and easily washable material)	may be required ~~no~~ / yes	~~may be required no / yes~~	~~required~~	~~required~~	
Floor and/or walls and ceiling easily washable	may be required ~~no~~ / yes	~~required for floor~~	~~required for floor and walls~~	~~required for floor, walls and ceiling~~	
Appropriate filters on isolators or isolated rooms (note 3)	not required	~~may be required no / yes~~	~~required~~	~~required~~	
Incinerator for disposal of animal carcasses	required to be accessible	~~required to be accessible~~	~~required to be accessible~~	~~required to be on site~~	
Appropriate barriers at the room exit, and at drains and ventilation duct work	required	~~required~~	~~required~~	~~required~~	
Animals kept in appropriate containment facilities, such as cages, pens, tanks or isolator	may be required ~~no~~ / yes	~~may be required no / yes~~	~~may be required no / yes~~	~~may be required no / yes~~	

CLASSIFICATION	CLASS 1	~~CLASS 2~~	~~CLASS 3~~	~~CLASS 4~~

Notes

1. "Animal unit" means a building, or separate area within a building, containing an animal facility and other areas such as changing rooms, showers, autoclaves, food storage areas etc.

2. "Animal facility" means a facility normally used to house stock, breeding or experimental animals or one which is used for the performance of minor surgical procedures on animals.

3. "Isolators" means transparent boxes where small animals are contained within or outside a cage; for large animals, isolated rooms may be more appropriate.

CONTAINMENT AND CONTROL MEASURES FOR GM ANIMALS NOT INFECTED WITH GMMS

In the following guidance, animal containment is divided into two categories. Containment A representing the minimum, or basic, recommended level of containment consistent with good practice. Containment B represents a higher category of containment.

ANIMAL CONTAINMENT A

Suitable activities

Containment A is recommended for GM animals which exhibit any of the following traits or properties:

- they are incapable of surviving in the environment in the UK, or;
- they have limited ability to transfer genetic material to UK animal species, or;
- female farm animals which are easily recalled, for example transgenic sheep, or;
- the genetic modification does not increase the level of risk to human health or the environment above that of the non-modified parental organisms;
- **and** the animals have not been inoculated with GMMs or other pathogens.

In all cases, Containment A is only suitable if the risk assessment, taking into account the animal, modification, activity and containment, is shown to be low or effectively zero. Examples of the types of GM animals for which this containment is appropriate are likely to include "knockout" mice; "nude" mice; tropical fish that are unlikely to survive in UK rivers; large mammals expressing pharmacologically active proteins in their milk, etc.

General Provisions

The following procedures and containment are recommended as minimum standards of good practice. They will need to be supplemented by measures for specific animal types. Annex I sets out recommendations for small mammals, large mammals, aquatic animals and invertebrates. The specific measures must be chosen in accordance with the risk assessment.

Minimum/baseline measures for Containment A:

- The containment areas for vertebrates and for *Octopus vulgaris* should be in accordance with Home Office requirements for animal welfare.
- Animals should be kept in appropriate containment, such as in animal rooms, or securely fenced areas, to minimise the possibility of accidental escape or theft.
- All potential routes of escape should be identified, and appropriate measures put in place to prevent egress. Mesh covering should be used to cover drains. The mesh size should be suitable to prevent the smallest animals escaping.
- The containment area should be kept locked, where appropriate, and monitored at frequent intervals.
- Animal barriers should be placed on exits from animal rooms to corridor areas when rooms or cages are being cleaned.
- A barrier should separate male and female animals, unless reproductive studies are part of the experiment, or unless other measures are taken to avoid sexual reproduction. Animals should be separated as soon as possible after weaning. Where it is difficult to determine the sex of an animal until sexual maturity, this should be carried out as early as possible. The use of reproductive incapacitation such as induction of triploidy in fish, may be used and if so must be covered in the risk assessment.
- A written record should be maintained of the experimental use and disposal of each animal or group of animals. The permanent marking of GM animals may be appropriate (depending on size), or alternatively the cages should be clearly labelled. For work with vertebrates, records required to be kept as part of a Home Office licence should provide sufficient detail.
- Animals should be transported to and from the facility in appropriate animal containers.
- Access to the containment facility should be restricted.
- A set of local rules should be produced, and these should be read by all staff using the facility.
- Staff should be given appropriate training and instruction on the procedures to be carried out.

ANIMAL CONTAINMENT B

Suitable activities

Containment B is recommended for GM animals which have any of the following characteristics:

- the animals could cause harm to humans or the environment if they escaped from the containment facility, **and** they have the ability to transfer novel genetic materials to UK animal species, or;
- the animals could establish outside of the containment facility, or;
- the genetic modification increases the level of risk to human health or the environment above that of the non-modified parental organisms;
- **and** the animals have not been inoculated with GMMs or other pathogens.

In all cases Containment B must be used when Containment A is insufficient to reduce all risks to low or effectively zero.

General Provisions

The following procedures and containment are recommended standards of good practice and should be applied in addition to the provisions of Animal Containment A. They will need to be supplemented by measures for specific animal types. Annex I sets out further specific recommendations which should be applied as appropriate and in accordance with the risk assessment.

In many cases the measures will be a more rigorous implementation of the requirements for Containment A, such as additional barriers, or more tightly controlled access.

In addition to the measures required for Containment A, the following procedures should be applied:

- Where small animals are being kept, floor drains should be avoided if possible. For older animal houses this may not be practicable, and double mesh barriers on drains should be used. These should be checked on a regular basis.
- Written operating procedures should be produced for all routine operations carried out within the facility.
- Staff should be given appropriate training and instructions on the procedures to be carried out, and written records of training should be kept.
- Written records of any accidents or escapes from cages or primary containment should be kept. Where any accident or escape could present an immediate or delayed hazard to human health the HSE must be notified forthwith.
- The containment area must be kept locked, and access tightly restricted. Security measures must be implemented to prevent theft or intentional release of animals through vandalism. Regular security patrols, and/or the use of closed circuit television may be appropriate.
- Discharge of water from tanks holding aquatic animals must not be direct to drain, but should pass through several filters. Regular checks should be made to ensure that filters are kept clean. If discharge into rivers or the marine environment is considered, additional protective measures should be implemented. No such discharge should take place without prior notification to HSE.

Annex 1

ADDITIONAL RECOMMENDATIONS FOR CONTAINMENT OF SPECIFIC GM ANIMAL TYPES

Small mammals (rodents eg. mice etc)

- Animals should be kept in appropriate cages. Cage sizes and minimum space requirements should be in accordance with Home Office rules. Cleaning procedures should be instituted which minimise the likelihood of escape.

- Floor drains and low level ventilation should be avoided, or made escape proof through the use of wire mesh or similar barriers.

- Appropriate rodent traps should be used.

- All animals should be tagged or marked, to allow individuals to be identified. Cages should be clearly marked.

- Experimental procedures, such as the administration of drugs and the bleeding of animals should be carried out in a way that minimises chance of escape.

Large mammals

- Animals should be kept in appropriate pens or fenced areas. Double fencing may be appropriate, dependent on the level of risk.

- Where the fenced area is in a remote location, or some distance from the main buildings, security measures should be taken to prevent theft or vandalism. Regular monitoring of the perimeter fencing, and/or the use of closed circuit television may be appropriate.

- In the case of animals that might burrow, the fencing should go down to a sufficient depth to minimise or prevent escape.

- Fencing should be of sufficient height and mesh size to prevent egress.

- Written records should be kept for all animals, including births, deaths and movement to the incinerator.

Aquatic animals

- Fish, including developing fertilised eggs should be kept in appropriate tanks. Tanks should have filters of sufficient mesh size to retain the smallest organism likely to be present. At the early stage of embryo development, the mesh size should be determined through knowledge of the variation in egg size.

- Secondary filters should be used to retain any eggs that may be detached from the hatching trays and also to prevent escape of fish throughout the life cycle; the number of secondary filters required will depend on the risk assessment.

- A cleaning regime should be instituted to prevent filter clogging. Filters should be checked regularly, particularly if water supply is from a river, or the sea, to prevent build up of algae or other material. Consideration should be given to the (sand) filtration of supply if build up of algae is identified as a major problem.

- High water alarms may be required depending on the risk assessment. It is likely that low water alarms may be used to protect stocks.

- Secondary containment may be required in case of overflow or rupture of vessels or tanks. Rupture or other damage may occur where people have to enter the tank to clean filters. Such procedures should be avoided where possible - for example, the use of long handled brushes may provide an alternative.

- The containment area may need to be bunded to prevent overflow to outside. This would

- Consideration should be given to the use of high water alarms at floor level, so that flooding is detected early. The alarm system should be audible and visible throughout the site and operate 24 hours a day.

- The use of a "soak away" outside the facility should be considered if the facility is close to a river or the sea. Such an area may consist of a gravelled area which allows water to soak through whilst retaining any escaped fish on the surface.

- Where an outlet pipe from a contained facility discharges directly into a river or the sea, extra care will need to be taken. Consideration should be given to the use of a filter barrier on the pipe into the final holding tank. An electrical kill system typical of the kind used in the aquaculture industry may provide a suitable final barrier before final discharge.

Invertebrates

- Appropriate cages or culture vessels should be used.

- The use of secondary containment measures, such as muslin "tents" should be considered if indicated by the risk assessment.

- Measures should be taken to enable escaped invertebrates to be detected and recaptured or destroyed. For ticks and mites, containers should be kept over trays of oil.

- All experimental cages/pens must be numbered and documented.

- Used culture vessels must be decontaminated before disposal or thoroughly cleaned before re-use.

- Flying or crawling arthropods should be handled on white trays to facilitate the detection of escape.

- The use of an electric insect control unit should be considered.

- The activity of the arthropod and the risk of accidental escape can be reduced by chilling, or keeping cages in cold rooms. The use of chilled corridors surrounding an insectory also helps prevent egress. place without prior notification to HSE.