Genetic Modification Safety

Objectives

- Legal requirements for work with genetically modified organisms
- Risk relating to genetically modified organisms at work
- Risk assessment and control for genetically modified organisms

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Risk Assessment

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GM Safety Law

- Health and Safety at Work Act and Regulations
- Environment Acts and Regulations
- Genetically Modified Organisms (Contained Use) Regulations
- Health and Safety Executive (HSE) regulator for biological safety

GM Risk Assessment and Control

- Responsibility of managers and principal investigators
- Assess risks to human health and environment
- Genetically modified organisms
- Activity
  - Who or what might be harmed and how
  - Activity class
  - Containment level and controls
  - Emergency procedures
  - Information, instruction, training and supervision
  - GMSC and HSE permission
  - Review and revise risk assessments

GM Safety Committee

University GMSC
- Chair, School GM Chairs, unions, occupational health physician and practitioner and biological safety officer
- Advise on GM risk assessment and controls, monitor activities and keep University records
- Permission system for genetically modified organisms

School GMSC
- School GM Chair and academic researchers
- Advise on GM risk assessment and controls, monitor activities and keep School records
Guidance and Information

Websites
- University Safety Office - Biological Safety
- University Occupational Health Service
- Health and Safety Executive
- Department for Environment, Food and Rural Affairs
- Health Protection Agency

Publications
- Microbiology and biology textbooks
- Scientific papers and internet searches

Hazards and Risks

Hazard
- Genetically modified organism

Risk
- Genetically modified organism and potential harm to humans and environment

Genetically Modified Organisms

Genetically modified organisms are organisms produced by genetic modification using a biological entity capable of replication of transferring genetic material and includes microorganisms but does not include humans, human embryos or human admixed embryos.
Genetic Modification

Altering genetic material of an organism in a way that does not occur naturally by mating or natural recombination or both

Contained Use Activity

Activity where organisms are genetically modified or where genetically modified organisms are cultured, stored, transported, destroyed, disposed of, or used in any way, and for which physical, chemical or biological barriers, or any combination, are used to limit contact of genetically modified organisms with humans and environment to ensure a high degree of safety

Genetically Modified Organism

GMO created by combining host, vector and genetic material

Host + Vector + Genes → Genetically modified organism
Genetically Modified Organisms

Genetically modified organisms (GMO) can be microorganisms, animals or plants

- Genetically modified microorganisms (GMM)
- Genetically modified animals (GM Animals)
- Genetically modified plants (GM Plants)

GMM can be nucleic acid (eg GM infectious virus DNA or RNA)

Exemptions

- Mutagenesis (eg x-rays, chemicals)
- Synthetic nucleic acids (non-hereditary)
- Humans and human embryos

Genetically Modified Organisms

- GM viruses
- GM bacteria
- GM fungi
- GM parasites
- GM cell cultures
- GM animals
- GM plants
- GM nucleic acid
GM Risk Assessment

1. University Safety Office website - GM Risk Assessment
2. Complete GM risk assessment form
3. Read and follow guidance (SACGM Compendium of guidance)
4. Contact GM Chair for advice
5. Permission for work from School GMSC, University GMSC and HSE

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Guidance

- SACGM Compendium of guidance
- ACDP Approved list of biological agents
- ACDP Biological agents: Managing risks in laboratories and healthcare premises

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SACGM Compendium of Guidance

- Part 1: Introduction to the legislation and general health and safety issues
- Part 2: Risk assessment of genetically modified microorganisms (other than those associated with plants)
- Part 3: Containment and control of activities involving genetically modified microorganisms
- Part 4: Genetic modification work that involves plants (including plant associated genetically modified microorganisms)
- Part 5: Genetic modification of animals
- Part 6: Guidance on the use of genetically modified microorganisms in a clinical setting

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GM Risk Assessment Forms

- GM risk assessment form 1 - Genetically modified microorganisms and animal cells excluding viruses
- GM risk assessment form 2 - Genetically modified human and animal viruses and viral vectors
- GM risk assessment form 3 - Genetically modified plant viruses
- GM risk assessment form 4 - Genetically modified plants
- GM risk assessment form 5 - Genetically modified animals
- GM risk assessment form - Generic E. coli
- GM risk assessment form - Minor modification to an approved GM project

GM Risk Assessment Form

- Section 1: Personnel
- Section 2: Project
- Section 3: Risk assessment for humans and environment
- Section 4: Controls
- Section 5: Emergency planning
- Section 6: Approval

Genetically Modified Organisms

GM Microorganisms
- Genetically modified microorganisms (GMM)

GM Animals
- Genetically modified animals (GM Animals)

GM Plants
- Genetically modified plants (GM Plants)
Classification of Genetically Modified Organisms

Genetically modified organisms are classified into four classes

- Ability to cause disease or harm to humans or environment
- Severity of disease or harm
- Likelihood disease or harm will spread
- Availability of effective prophylaxis or treatment

<table>
<thead>
<tr>
<th>Activity class</th>
<th>Description</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Activity class 1 (Class 1)</td>
<td>Lowest risk</td>
<td>HG1 Biological agents (Minimum for host) E. coli K12 or BL21 strains and derivatives with harmless genes</td>
</tr>
<tr>
<td>Activity class 2 (Class 2)</td>
<td>Lowest risk</td>
<td>Harmless replication defective virus vectors with harmless genes</td>
</tr>
<tr>
<td>Activity class 3 (Class 3)</td>
<td>Middle risk</td>
<td>Replication defective vectors or competent HG2 viruses with harmless or harmful genes</td>
</tr>
<tr>
<td>Activity class 4 (Class 4)</td>
<td>Highest risk</td>
<td>HG3 Biological agents (Minimum for host)</td>
</tr>
</tbody>
</table>

SACGM Activity Classes

Class | Description | Examples |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Unlikely to cause human disease or environmental damage</td>
<td>HG1 Biological agents (Minimum for host) E. coli K12 or BL21 strains and derivatives with harmless genes</td>
</tr>
<tr>
<td>2</td>
<td>May cause human disease but unlikely to cause significant environmental damage</td>
<td>HG2 Biological agents (Minimum for host) Replication defective vectors or competent HG2 viruses with harmless or harmful genes</td>
</tr>
<tr>
<td>3</td>
<td>May cause severe human disease or significant environmental damage</td>
<td>HG3 Biological agents (Minimum for host)</td>
</tr>
</tbody>
</table>

Rules for Activity Class 1

Possession or use of Class 1 genetically modified organisms

- GM risk assessment
- School GMSC and University GMSC advice and approval
- Implement and monitor controls
- Review and revise GM risk assessment with advice and approval from School GMSC and University GMSC
- Keep all records
**Rules for Activity Class 2**

Possession or use of Class 2 genetically modified organisms

- GM risk assessment
- School GMSC and University GMSC advice and approval
- HSE notification, advice and approval
- Implement and monitor controls
- Review and revise GM risk assessment with advice and approval from School GMSC, University GMSC and HSE
- Keep all records

**Rules for Activity Class 3**

Possession or use of Class 3 genetically modified organisms

- GM risk assessment
- School GMSC and University GMSC advice and approval
- HSE notification, advice and approval
- Implement and monitor controls
- Review and revise GM risk assessment with advice and approval from School GMSC, University GMSC and HSE
- Keep all records

**New GM Risk Assessment**

- PI completes GM risk assessment
- Email based system
- Email GM risk assessment to School GM Chair
- GM Chair advises PI, approves GM risk assessment and sends to University GMSC
- University GMSC advises PI and approves GM risk assessment
- For Class 2 and 3 University GMSC advises PI and sends notification to HSE
- HSE advice and consent
- University GMSC sends approval certificate to PI
Changes to GM Risk Assessment

- PI completes GM risk assessment minor modification
- Email based system
- Email GM risk assessment minor modification to School GM Chair
- GM Chair advises PI, approves GM risk assessment minor modification and sends to University GMSC
- University GMSC advises PI and approves GM minor modification
- University GMSC approves GM risk assessment minor modification
- University GMSC sends approval certificate to PI

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Closure of GM Risk Assessment

- PI requests permission to close GM risk assessment
- Email based system
- Email request to School GM Chair
- School GMSC and University GMSC advise PI and approves closure
- Can not close a GM risk assessment and keep GMO
- All GMO must be destroyed or transferred to a suitable GM risk assessment
- University GMSC sends approval to PI

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HSE Notification of GM Activities

HSE notification is required for Class 2 and 3 GM activities

- GM risk assessment
- HSE CU2 form
- HSE fee
- BSO sends GM risk assessment and CU2 form to HSE
- PI pays fee to HSE
- HSE response, advice, questions, consent or refusal

HSE notification and fee is not required for Class 1 GM activities

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HSE Notification

- First contained use any Class GMM
- First use Class 2 GMM (45 day notice)
- First use Class 3 GMM (90 day notice and Consent)
- Subsequent use Class 2 GMM (Acknowledgement)
- Subsequent use Class 3 GMM (45 day notice and Consent)
- First or subsequent use harmful GM animal or GM plant (45 day notice)
- Can notify a connected scheme of work of several risk assessments in single notification
- Can apply for derogations (Permission to use lower containment level or less stringent controls than usually required)

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HSE Notification

<table>
<thead>
<tr>
<th>Subject</th>
<th>Fee</th>
<th>Form</th>
<th>Risk assessment</th>
<th>Notification period</th>
<th>Consent</th>
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<tbody>
<tr>
<td>Class 2 GMM</td>
<td>£929</td>
<td>CU2</td>
<td>Yes</td>
<td>None</td>
<td>No</td>
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<tr>
<td>Class 3 GMM</td>
<td>£1007</td>
<td>CU2</td>
<td>Yes</td>
<td>45 days</td>
<td>Yes</td>
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<tr>
<td>Harmful GM animal or plant</td>
<td>£929</td>
<td>CU2</td>
<td>Yes</td>
<td>45 days</td>
<td>No</td>
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<tr>
<td>Significant change to risk assessment</td>
<td>£996</td>
<td>Letter</td>
<td>Yes</td>
<td>None</td>
<td>Variable</td>
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<tr>
<td>Derogation with notification</td>
<td>£0</td>
<td>CU2</td>
<td>Yes</td>
<td>N/A</td>
<td>Variable</td>
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<tr>
<td>Derogation after notification</td>
<td>£996</td>
<td>Letter</td>
<td>Yes</td>
<td>N/A</td>
<td>Variable</td>
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</table>

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Notification of Class 2 Activities

- Class 2 GM risk assessment
- PI and BSO complete CU2 form
- BSO sends GM risk assessment and CU2 to HSE
- PI pays HSE £929 by BACS
- HSE response (eg Request more information, reconsider hazards, risks or controls)
- PI must address HSE questions (eg Provide more information on hazards, risks or controls, amend risk assessment)
- BSO response to HSE
- HSE consent or refusal
- Notify future significant changes

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**Notification of Class 3 Activities**

- Class 3 GM risk assessment
- PI and BSO complete CU2 form
- BSO sends GM risk assessment and CU2 fee to HSE
- PI pays HSE £1007 by BACS
- HSE response (e.g., request more information, reconsider hazards, risks or controls)
- PI must address HSE questions (e.g., provide more information on hazards, risks or controls, amend risk assessment)
- BSO response to HSE
- HSE consent (rarely refusal)
- Notify future significant changes

**Risks to Human Health and Environment**

- How and to what could people or environment be exposed
- What harm to humans or environment
- Route and consequence of exposure or release
- Change to pathogenicity, toxicity, carcinogenicity, cell tropism or host range
- Virulence factors, toxins, immunomodulators, oncogenes, antibiotic or biocide resistance
- Transfer of genetic material to other organisms
- Spread to close contacts or community
- Release, survive, spread or displace species in environment
- Could harm be treated or remedied

**Risks to Human Health and Environment**

<table>
<thead>
<tr>
<th>Hazards</th>
<th>Hazards</th>
<th>Hazards</th>
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</thead>
<tbody>
<tr>
<td>Host</td>
<td>Host</td>
<td>Host</td>
</tr>
<tr>
<td>Vector</td>
<td>Vector</td>
<td>Vector</td>
</tr>
<tr>
<td>Insert genes</td>
<td>Insert genes</td>
<td>Insert genes</td>
</tr>
<tr>
<td>Final GMO</td>
<td>Final GMO</td>
<td>Final GMO</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Risks to humans</th>
<th>Risks to environment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workers</td>
<td>Microorganisms</td>
</tr>
<tr>
<td>Visitors</td>
<td>Animals</td>
</tr>
<tr>
<td>Public</td>
<td>Plants</td>
</tr>
<tr>
<td>Other people</td>
<td>Food</td>
</tr>
</tbody>
</table>
**Release or Exposure Routes**

- Inhalation, ingestion, injection or absorption
- Sharps injuries, wounds, animal bites or scratches
- Release of GMM, GM animals or GM plants
- Release of animals or plants infected with GMM
- Release of vector (e.g., retroviral vector)
- Release of pollen or other plant propagules
- Release by land, air, water, sewage or waste
- Spread by insect or pest vectors
- Spillages (e.g., drains) and aerosols
- Breakage and exposure or release during transport
- Incorrect inactivation or disposal of GMO

**GMO Risks**

Risks of all components of GMO must be assessed

- Host
- Vector
- Genetic material
- Final GMO (GMM, GM animal or GM plant)

**Hosts**

Host organism is basic component of GMO

- Microorganisms (e.g., E. coli, S. cerevisiae, retroviruses, human, animal and plant cell cultures)
- Animals (e.g., C. elegans, D. melanogaster, M. musculus and r. norvegicus)
- Plants (e.g., A. thaliana, Z. mays, N. tabacum and T. aestivum)
Vectors

Vectors for gene cloning, propagation, manipulation, transfer, mutagenesis and expression of genetic material

- Cloning, expression and shuttle vectors
- Replication defective adenovirus vectors
- Replication defective retrovirus vectors (e.g., lentivirus vectors)
- Replication competent disabled virus vectors
- Replication competent viruses (e.g., wild type, attenuated or virulent)

Genetic Material

Genetic material could be any coding or non-coding DNA or RNA sequence

- **Harmless**: Majority of genes in an organism
- **Harmful**: Minority of genes in an organism (e.g., virulence, toxins, oncogenes, immune modulators)
- **Undefined**: Function unknown

Genes which are harmless in one context or species might be harmful in different context or species

Risk Assessment of GM Microorganisms and Animal Cells

- GM risk assessment form 1 - GMM and animal cells
- Pathogenic or non-pathogenic bacteria, fungi, parasites, human or animal cell cultures
- Cancer cell lines, human or animal stem cells
- Genetic modification of harmful genes affecting virulence, toxicity, carcinogenicity, immunity, tissue tropism or host range
- Gene libraries (e.g., harmless and harmful genes)
- Accidental release
- Phage, plasmid or chromosomal mediated transfer
- Transfer of genes to same or other species in environment
- Survival of GMM outside containment in environment
**Escherichia coli K12 & BL21**

- Highly researched microorganism used in genetics and molecular biology
- *E. coli* strains are hazard group 2
- *E. coli* K12 and BL21 strains and derivatives are multiply mutant and highly disabled
- Long history of safe use
- *E. coli* K12 and BL21 are hazard group 1
- Class 1 GMO
- Genetic engineering work with microorganisms, cell cultures, animals and plants

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**Risk Assessment of Generic *E. coli***

- GM risk assessment form - Generic *E. coli*
  - Includes
    - *E. coli* K12 strains and derivatives
    - Harmless eukaryotic genomic or cDNA genetic material
  - Excludes
    - *E. coli* BL21 strains and derivatives
    - Prokaryotic genetic material
    - Gene expression
    - Harmful eukaryotic genomic or cDNA genetic material
    - Genetic material known or suspected to be oncogenic, virulent, toxic, allergenic or detrimental if delivered to a target tissue

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**Risk Assessment of GM Human and Animal Viruses and Viral Vectors**

- GM risk assessment form 2 - GM viruses and viral vectors
- Replication competent or defective viruses and viral vectors
- Pathogenic or non-pathogenic human or animal viruses
- Modification of cell tropism or host range (eg VSV-G)
- High transfection efficiency of human and animal cells
- High virus titres, target dividing and non-dividing cells
- Harmful genes in viruses or viral vectors
- Powerful gene expression regulators
- Transmission, mobilisation or activation of oncogenes
- Recombination of viruses and viral vectors
- Insertional mutagenesis and permanent modification of host chromosomes
Risk Assessment of GM Plant Viruses

- GM risk assessment form 3 - GM plant viruses
- Replication competent or defective viruses and viral vectors
- Accidental release
- Release of experimentally infected plants
- Transfer of genes to species of plants in environment
- Harmful genes in viruses or viral vectors
- Modification of cell tropism or host range
- Transmission, mobilisation or activation of oncogenes
- Insertion of powerful gene expression regulators
- Recombination of viruses and viral vectors
- Insertional mutagenesis and permanent modification of host chromosomes

Risk Assessment of GM Plants

- GM risk assessment form 4 - GM plants
- Plants, plant vectors (e.g. A. tumefaciens) and plant cell cultures
- Non-GM or GM plants experimentally infected with GMM
- Accidental release (e.g. pollen, seeds, somatic parts)
- Release of experimentally infected plants
- Transfer of genes to same or other species of plants in environment
- Cross fertilise other plants in environment
- Survival of GM plant outside containment in environment

Risk Assessment of GM Animals

- GM risk assessment form 5 - GM animals
- Non-GM or GM animals experimentally infected with GMM
- Accidental release (e.g. flying, crawling, walking or swimming)
- Release of experimentally infected animals
- Transfer of genes to the same or other species of animal in environment
- Breed with animals in environment
- Survival of GM animal outside containment in environment
Risk Assessment of Minor Modifications to GM Projects

- GM risk assessment form - Minor modification to approved GM project
- Change to project (Hosts, vectors and genetic material)
- Change to personnel (Names, start and end dates)
- Change to location (Buildings and room numbers)
- Change to closing date
- Closure of GM risk assessment
- All changes to approved GM risk assessments must be made using GM minor modification form

Creation of Novel GMO Risks

Could your GMO create novel risks to humans, animals, plants or other aspect of environment?

- Existing or known risks
- Intentional creation of novel risks
- Unintentional creation of novel risks
- Potential dangers of some example GMO experiments

Novel GMO Risks

- Replication defective adenovirus expressing cytokine IL-1 (Transient expression sufficient for acute lung injury and pulmonary fibrosis)
- Replication competent Mousepox virus expressing IL-4 (Modification of host immune response and hypervirulence)
- Regulatory gene deletion mutants of Mycobacterium tuberculosis (Knockout mutation and hypervirulence)
- Replication competent adenovirus expressing SV40 large T gene (Aerosol transmission of potentially oncogenic virus)
- Murine moloney leukaemia virus expressing VSV envelope protein (Expanded host range and tissue tropism)
- Insertion of Bacillus anthracis hemolysin gene into Bacillus cereus (Confers virulence factor and increased pathogenicity)
Hypervirulent GM Mousepox-IL4 Virus

- Genetic engineering of mousepox virus for vaccine to control reproduction of rodents and rabbits
- GM mousepox virus expressing interleukin 4
- Expression of cytokine genes to enhance antibody immunity
- High mortality of infected mice even of previously vaccinated mice and highly genetically resistant strains
- IL4 suppressed cellular immunity and memory
- CMI major defence against intracellular pathogens
- Hypervirulent strain of highly pathogenic GM virus
- Mousepox does not normally infect humans
- GM mousepox infection of workers or escape from lab?

Hypervirulent GM M. tuberculosis

- Mycobacterium tuberculosis mutation screen to identify genes involved in pathogenicity
- Screen of GMO deletion mutants to identify virulence genes
- Single gene deletion M. tuberculosis clones used to infect mice
- Deletion mutations in most genes reduced virulence
- Deletion mutation in one gene resulted in hypervirulent phenotype
- Hypervirulent strain (eg mice, animals, humans?)
- Gene identified as global negative regulator of virulence gene expression
- GM M. tuberculosis infection of workers or escape from lab?

Rescue of 1918 Pandemic Influenza Virus

- 1918/19 approx 50-100 million people died of Influenza virus A/H1N1/1918
- Influenza A 1918 virus RNA isolated from fixed and frozen pathological human lung tissue samples, RT-PCR and sequenced in USA
- Recombinant Influenza A virus 1918 rescued using reverse genetics from DNA clones in cell culture in USA
- Virus used for research in cell culture and animals
- Containment level 3 (Sufficiently high?)
- Risk of infection of workers?
- Risk of escape from lab?
**When Things Go Wrong**

- Sometimes things do go wrong
- GM adenovirus vector fatality
- GM retrovirus vector fatalities
- GM vaccinia virus infections

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**GM Adenovirus Vector Fatality**

- Clinical trial using GM adenovirus vector for treatment of liver genetic disorder patient in 1999
- Patients exposed to large doses of GM adenovirus in therapy
- Individual deteriorated, comatose, developed acute respiratory distress syndrome and died of multiple organ failure due to anoxia
- Autopsy found severe reduction in precursor white cells in bone marrow
- Measurements of cytokines showed vector caused systemic inflammatory response syndrome associated with ARDS
- GM adenovirus injected into liver but found in other organs
- Other people on trial of GM adenovirus therapy were fine

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**GM Retrovirus Vector Fatalities**

- Clinical trial using GM retrovirus vector for treatment of SCID genetic disorder patients in 2003
- 11 “bubble boys” treated in France for immune disease X-SCID
- 2 developed leukaemia and died
- Mouse GM retrovirus used to transfer healthy copy of gene to bone marrow cells
- In some cells DNA integrated near gene that can trigger cancer if disrupted
- Retroviruses which carry their own protein tools to splice themselves into chromosomes often integrate at hotspots near certain active genes
GM Vaccinia Virus Infections

- GM vaccinia virus strain infected laboratory worker in 2002
- Individual was hospitalised with unknown infection which was identified as vaccinia but recovered
- Trained in laboratory techniques and risks of working with vaccinia virus
- Not aware of signs of vaccinia infection and did not realise she had been infected
- Many GM vaccinia virus infections of lab workers recorded in vaccinated and unvaccinated individuals and close contacts
- Often inadequate information, training and supervision
- May cause severe disease and death during eczema or psoriasis, pregnancy or immunocompromised such as HIV

Risk Estimation

Risk is estimated by combining severity of harm were it to occur and likelihood of occurrence in specific circumstances

- Severity of harm (severe, moderate, minor, negligible)
- Likelihood of harm (high, medium, low, negligible)

Risk = Likelihood \times Severity

Risk = Effectively zero, Low, Low/Medium, Medium or High

Risk Estimation Matrix

<table>
<thead>
<tr>
<th>Severity of Harm</th>
<th>Likelihood of Harm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>High</td>
</tr>
<tr>
<td>Severe</td>
<td>High</td>
</tr>
<tr>
<td>Moderate</td>
<td>High</td>
</tr>
<tr>
<td>Minor</td>
<td>Medium/Low</td>
</tr>
<tr>
<td>Negligible</td>
<td>Effectively zero</td>
</tr>
</tbody>
</table>
Control of Genetically Modified Organisms

- Protection of humans and environment requires effective containment and control
- Human and environmental exposure to genetically modified organisms must be prevented or adequately controlled
- Exposure to genetically modified organisms must be reduced to a level which is adequate to protect humans and environment

Containment and Control

- Policies, risk assessments and standard operating procedures
- Containment laboratories and controls
- Controls for GM Microorganisms, GM animals and GM plants
- Biological controls
- Safe work practices for use, storage, transport, inactivation and waste disposal
- Hygiene
- Personal protective equipment
- Health surveillance
- Emergency plans and procedures
- Information, instruction, training and supervision
### Containment and Control

1. Select containment level
2. Select controls required for containment level
3. Select additional controls required in risk assessment
4. Select most effective controls using hierarchy of risk control
5. Controls must be proportionate to risks

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### Containment Levels

Minimum containment level required is equivalent to activity class

- Containment level 1 (CL1) for activity class 1 (Class 1)
- Containment level 2 (CL2) for activity class 2 (Class 2)
- Containment level 3 (CL3) for activity class 3 (Class 3)
- Containment level 4 (CL4) for activity class 4 (Class 4)

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### Containment Laboratories

Containment levels required for general, animal and plant laboratories

- CL1 for low risk work with class 1 genetically modified organisms
- CL2 for medium risk work with class 2 genetically modified organisms
- CL3 for high risk work with class 3 genetically modified organisms

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Containment Level 1

- Suitable for low risk activity class 1 work
- Access limited to authorised persons
- CL1 sign
- Benches, floors and walls impervious, resistant and cleanable
- Autoclave and effective disinfectants
- Hand wash sink with emergency wash hose
- Appropriate PPE (e.g. lab coats, gloves, specs etc)

Containment Level 2

- Suitable for medium risk activity class 2 work
- Access to authorised persons only
- CL2 and biological hazard signs
- Benches, floors and walls impervious, resistant and cleanable
- Negative pressure ventilation
- Safety cabinet, isolator or containment used for infectious aerosols
- Autoclave and effective disinfectants
- Hand wash sink with emergency wash hose
- Appropriate PPE (e.g. lab coats, gloves, specs etc)

Containment Level 3

- Suitable for high risk activity class 3 work
- Access to authorised persons only
- CL3 and biological hazard signs
- Benches, floors and walls impervious, resistant and cleanable
- Laboratory must contain own equipment
- Negative pressure ventilation and exhaust air HEPA filtered
- Safety cabinet, isolator or containment used for infectious aerosols
- Autoclave in laboratory and effective disinfectants
- Laboratory must be sealable to permit fumigation
- Hand wash sink with emergency wash hose
- Appropriate PPE (e.g. gowns, gloves, specs etc)
### Basic Controls for GMM

- Containment laboratory
- Safe use, storage, transport, inactivation and waste disposal
- Biological controls
- Safety cabinets
- Dedicated equipment and PPE
- Access control and locked rooms

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### Basic Controls for GM Animals

- Containment laboratory
- Safe use, storage, transport, inactivation and waste disposal
- Biological controls
- Safety cabinets
- Dedicated equipment and PPE
- Access control and locked rooms
- Isolators and individually ventilated cages
- Pest and vector controls
- Home Office licences for animal welfare
- DEFRA licences required for specific animal pathogens and pests and animals

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### Basic Controls for GM Plants

- Containment laboratory
- Safe use, storage, transport, inactivation and waste disposal
- Biological controls
- Safety cabinets
- Dedicated equipment and PPE
- Access control and locked rooms
- Isolators and propagators
- Removal or bagging of flowers, pollen and seeds
- Pest and vector controls
- DEFRA licences required for specific plant pathogens and pests and plants

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### Biological Controls

- Microorganisms, animals and plants with reduced capacities
- Disabled mutant strains (e.g., *E. coli* K12, retroviral vectors)
- Attenuated strains (e.g., vaccine strains)
- Host range modified mutant strains
- Reduced replication capacity
- Non-colonising mutant strains
- Non-transmissible vectors or genetic elements
- Non-mobilisable vectors or genetic elements
- Partial or non-expressed gene sequences
- Tightly regulated expression
- GMO with multiple disabling mutations (host, vector and genetic material)

### Inactivation of GMO

- Physical or chemical methods used to kill genetically modified organisms
- Autoclaving
- Disinfection
- Validation and monitoring of effectiveness is required to prove it works
- Follow manufacturers instructions
- Effectiveness of inactivation affected by many factors (e.g., species, time, temperature, pH, concentration, humidity, organic matter)
- Problems with inactivation of mixed waste (e.g., biological agents, chemicals, radiation)

### Disinfection

- Disinfectant must be suitable for GMO
- No universal disinfectant
- Narrow or broad spectrum activity
- Variable and unreliable
- Disinfectants are harmful
- Use PPE
- Dilute accurately and discard when inactive
- Disinfectant absorbent granules useful for spillages
- Check manufacturers validation of effectiveness
- Disinfection not reliable for inactivation of GMO
Autoclaving

- Autoclaving is most effective method for inactivating genetically modified organisms
- Standard 121°C or 134 °C for 15-30 minutes
- Validation of effectiveness using annual thermocouple testing is required
- Monitoring of effectiveness using electronic probes and recorders or chemical indicators is required
- Do not autoclave GMO hazards containing radioactive or hazardous chemical substances

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Autoclave Validation and Monitoring

- 'All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored by continuous chart (or digital) recording of the temperature/time profile'

- 'All contaminated materials, including waste destined for incineration, will be inactivated by autoclaving (100% kill) prior to disposal of waste or cleaning and recycling of reusable laboratory equipment, such as glassware. Autoclaves will be validated by annual thermocouple mapping and each run will be monitored using TST (Time, Steam, and Temperature) test strips (Albert Browne Ltd., TST class 6 emulating indicator 121ºC for 20 min)'

Any questions?

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