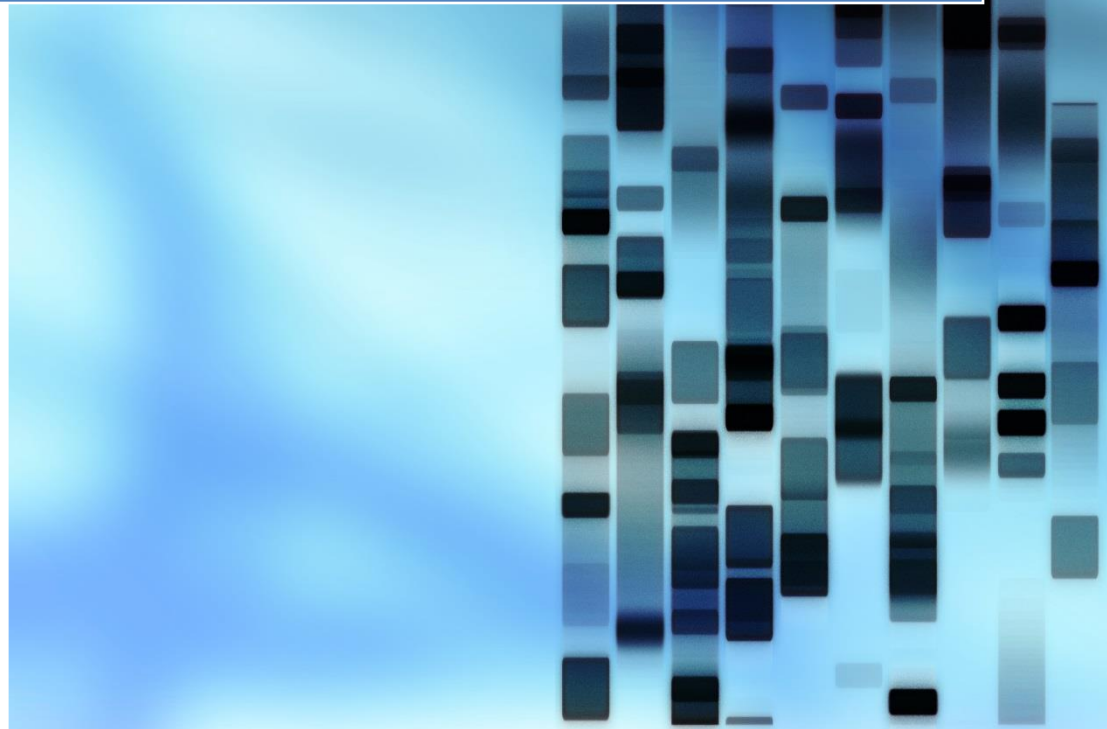


Genetically Modified Organisms (Contained Use) Regulations 2014: Significant change: guidance by example



Institute of Safety in
Technology and Research

Significant change – guidance by example

Background

1. The Genetically Modified Organisms (Contained Use) Regulations contain various duties that relate to the notification of contained use involving genetic modification including the need to inform the competent authority of any change to an existing notification. In particular, any significant change likely to affect the risks associated with the notified contained use¹.
2. Administrative changes such as changes to addresses, addition of new premises are relatively straight forward. Experience has shown however that the interpretation of what is meant by a significant change, ie where the risk(s) of the notified contained use has changed, has presented challenges to the biosafety community.
3. The Institute of Safety in Technology and Research (ISTR) Biosafety Steering Group, in consultation with the Health and Safety Executive (HSE) and with input from the UK biosafety community (via the regional BSO network), have prepared the following guidance to supplement the official HSE guide to the GMO (CU) Regulations 2014. There is no legal requirement to follow this guidance, although duty holders may find it helpful when considering the requirements under regulation 15 of the GMO (CU) Regulations.
4. ISTR serves safety professionals in technology and research including education, industry, government agencies and consultancy. The aim of the Institute is to enhance the knowledge, competence and professional development of its members through networking, accreditation, knowledge exchange, workshops and symposia, communication with regulators and partnerships with other safety organisations. The ISTR BSG represents the interests of UK biosafety nationally and internationally on behalf of ISTR.

HSE guidance

5. The formal guide to the regulations states that:

Where changes are necessary to the ongoing contained use, regulation 15 requires the notifier to inform the competent authority where these changes are deemed to be 'significant', specifically where these changes increase or present different risks from the notified work. Risk means risk to human health or the environment in the case of contained uses involving GMMs, but for contained uses involving larger GMOs it means risk to human health only. 'Significant' changes are any proposed modification to the ongoing work or where new information emerges that changes the rationale upon which the risk assessment is based. These changes are significant if they lead to the user having to change the way they work (eg, containment or control measures) or they present different or increased hazards/risks to those undertaking the work (eg, inherent properties of the GMM). Many of the changes associated with ongoing contained uses will not meet these criteria but rather will involve alterations having little or no effect on the hazards or risks associated with the

¹ The term activity is no longer defined separately in the 2014 regulations; instead the term "contained use" is used throughout the regulations, the guide to the regulations and consequently this document also.

work. In such circumstances, it is acceptable for these changes to be dealt with through the local risk assessment process, without notification to the competent authority.

6. HSE have identified a number of types of changes that might require notification (see Table 1); however the decision to notify is for the duty-holder themselves to determine.

Table 1: Types of change

Type of change		Guidance
1	Changes to containment and control measures	Risk based need to implement additional controls (within same containment level) e.g. small to large scale culture volume, necessitating applying the containment measures in Table 2 of Schedule 8; Changes to procedures such as different research procedures/techniques that increase the risks to users (e.g. type of filtration; centrifugation; use of sharps)
2	Use of different organisms or strains of organisms with different inherent characteristics	Relevant characteristics include route of transmission, pathogenicity, tropism, availability of treatment or prophylaxis: this would include moving from attenuated to virulent strains (e.g. replacement of vaccine strains, replication incompetent strains, strains with different host range)
3	Use of different vector, recipient organisms or genetic inserts for GM work	Using inserts with more harmful properties/extending host range; using vectors with fewer disabling mutations; using a host organism able to persist in the environment
4	Change in nature of the work	Moving from <i>in vitro</i> to <i>in vivo</i> work; changing the <i>in vivo</i> model (e.g. mice to birds) being studied
5	Changes to any consent conditions	For Class 3 and Class 4 contained use, consents may have conditions attached to them (e.g. derogation of control measures, limits of the scope of the work)
6	New information emerges that changes the consequences of exposure	New information may be from scientific literature or preliminary research findings, which affects the rationale upon which the risk assessment for the work is based (e.g. virulence function ascribed to previously unknown gene; research demonstrates more severe pathogenicity than envisaged at the outset)

Guidance by example

7. To help guide the decision making process, members of the UK biosafety community have provided a number of examples of actual changes to contained use from their organisations that were deemed significant (and in some cases not significant).

8. These examples have been anonymised and it should be noted that the decisions taken were on the basis of information available at the time. Each example is presented in the form of a short summary of the:

- Original outline of the work
- Proposed amendment(s)

- Conclusion of review of changes – reviews may have been carried out by the local Genetic Modification Safety Committee, the organisation’s Biological Safety Adviser/Officer, the user or a combination thereof.

9. Table 2 maps the information provided against the types of change identified by HSE, with links to summaries of the change.

Table 2: Examples of change/no change

Type of change		Significant change	Not a significant change
1	Changes to containment and control measures	1 , 4 ² , 6	
2	Use of different organisms or strains of organisms with different inherent characteristics	7	5
3	Use of different vector, recipient organisms or genetic inserts for GM work	8	3
4	Change in nature of the work	1 , 2 , 6 , 7	
5	Changes to any consent conditions	7	
6	New information emerges that changes the consequences of exposure	6	

² This example has 2 changes, one of which was not deemed significant albeit it involved a change in the range of viruses under study.



Example 1: Induction of immune responses in cattle using Modified Vaccinia Ankara (MVA) expressing mycobacterial antigens

Class 2 contained use

Outline of the Work

The aim of this work was to assess the potential of new vaccination strategies in protection against bovine TB using MVA virus expressing immunodominant mycobacterial antigens. These were to be expressed from a single promoter as a single fusion protein of 'Ag85A, TB10.4, TB9.8 and Acr2 (although not known to be toxic, carcinogenic or virulence factors themselves, they are known to be expressed from a region of the genome present only in virulent *Mycobacteria* and Acr2 particularly may cause immune-modulatory effects). Cattle, held under Containment Level 2 conditions, were to be vaccinated the BCG (prime) and boosted at defined intervals with the MVA expressing immunodominant antigens from *Mycobacteria*. Cattle were to be inoculated via the intramuscular route to minimise shedding. Control measures stated that staff handling the animals should wear protective gloves and disposable suits.

Proposed changes to GM contained use

To change the route of administration of the MVA expressing mycobacterial antigens to the cattle; specifically to administer the inoculation via the intranasal and intratracheal routes in order to assess different vaccination strategies.

Conclusion

Following review by the GMSC, it was acknowledged that the new routes of administration will increase the risk of exposure to the GMOs by the aerosol route during/after the administration procedure and that additional control measures such as the wearing of FFP3 RPE and eye protection would be required.



Example 2: Connected Programme Work: Recombinant Foot and Mouth Disease Virus (FMDV) Research

Class 4 contained use

Outline of the Work

Projects under the connected programme comprised studies looking at the mutagenesis of:

- FMDV sequences involved in RNA replication which required the use of infectious FMDV cDNA
- FMDV capsid sequence
- FMDV sequences involved in RNA replication which required the uses of FMDV replicon and structurally modified master seed viruses to enhance conventional FMDV vaccine protection.

All work was originally notified as a Class 4 contained use. All studies were *in vitro* based assays with propagation in tissues culture only.

Proposed changes to GM contained use

Following characterisation of the FMDV *in vitro* expressing epitope tags and fluorescent marker proteins, it was proposed to move into *in vivo* work and administer recombinants expressing marker proteins and epitope tags, via differing routes, to various animal species including mice, ruminants and pigs.

Conclusion

Following review by the GMSC, this was deemed as a significant change as the original scope of the connected programme did not extend to *in vivo* studies. The proposed work remained as a Class 4 contained use.



Example 3: Studies of physiology and pathogenicity of the *Mycobacterium tuberculosis* (Mtb) complex

Class 3 contained use

Outline of the Work

The work involved creating various strains of Mtb including deletion mutants, complemented strains, reporter strains and overexpressing specific genes in order to study the pathogenicity of Mtb. Further, some of the isolates used are clinical. The scope of the original notified contained use allowed changes to any Mtb open reading frame (ORF) or non-coding Mtb DNA region, and the use of non-Mtb homologues to Mtb ORFs.

Proposed changes to GM contained use

It was proposed to expand the contained use to include 10 additional vectors, the use of an additional marked mutant using other antibiotic resistance markers that were not originally defined, the use of epitope tags, and controlling gene expression using controllable promoter sequences. Waste procedures were also changed as the first line disinfectant was removed from the market.

Conclusion

The GMSC raised concerns that the additional antibiotics could be used in human therapy. However none of the antibiotics used are defined first or second line antibiotics used in the treatment of Mtb. Overall, the risk levels had not changed and the group would still be operating under containment level 3. As an amendment to a notifiable project, the GMSC's default position was to notify the changes to the HSE. However, advice was sought first from the HSE who felt that the changes did not affect the consequences arising from the work in a significant way.



Example 4: Investigating host and viral genetic variation and the effect of such variation on viral pathogenesis in vitro and in vivo

Class 2 contained use

Outline of the Work

This was notified as a connected programme of work with the aim of investigating how genetic variation in the host (gene mutation, gene expression differences, gene deletion and gene duplication) and in medically important human viruses affects the host response to infection, virus virulence and pathogenesis. The viruses under investigation are all classified as Hazard Group 2 biological agents and include:

- human herpesviruses, including Kaposi's sarcoma associated herpesviruses (KSHV), Epstein Barr Virus (EBV), and Herpes Simplex Virus (HSV-1), both wild type viruses and viruses derived from recombinant bacterial artificial chromosomes
- murine herpesviruses, including murine herpesvirus 68
- human orthomyxovirus, influenza viruses
- human paramyxovirus, measles virus
- lentiviral vectors

Proposed changes to GM contained use

It was proposed to extend the range of species being studied to include strains of Influenza B, where only Influenza A was notified previously and to introduce the culturing of influenza virus by inoculation of embryonated chicken eggs.

Conclusion

Extending the species being studied was not considered significant because the mode of transmission, host range, infectivity, and sensitivity to physical and chemical inactivation methods are equivalent. However use of eggs will necessitate the use of sharps, in the form of needles, to inject virus into the eggs. As there is no means of eliminating this specific use of sharps, the process will be performed in accordance with a strict SOP by experienced staff ie measures will be taken to control exposure. Therefore, this was considered a significant change since the control measures would change from those originally notified.



Example 5: DNA modification of selected Gram positive and negative bacteria

Class 2 contained use

Outline of the Work

This was notified as a connected programme of work with the aim of developing novel antimicrobial strategies to combat infections caused by selected Gram positive and negative bacteria. This will be achieved by disrupting genes on the chromosome and determining their role in growth, survival, stress resistance and interactions with host tissues and cells. Complementation of the mutation will follow mutagenesis in an effort to restore wild-type phenotype. Additionally reporter constructs will be made in order to determine the transcriptional and translational effects of mutant genes, environmental stimuli and growth phase on expression of genes involved in virulence and pathogenesis.

Proposed changes to GM contained use

To increase the number of agents under study (both as sources of genetic material and as host organisms) – additional agents shown in second column of table:

Original agents	Additional agents
The firmicutes; (majority of which are classed as group 2 by the ACDP. Do not propose to use any organisms which are above group 2).	Acinetobacter spp
Campylobacter jejuni	Peptostreptococcus spp
Helicobacter pylori	Vibrio cholerae (excluding El Tor)
The Pasteurellaceae	Vibrio spp
Porphyromonas gingivalis	Yersinia enterocolitica
Prevotella intermedia	Yersinia pseudotuberculosis
Escherichia coli	Campylobacter spp
Pseudomonas aeruginosa	Moraxella catarrhalis
Veillonella parvula, V. dispar	Burkholderia cepacia
Propionibacterium acnes	Klebsiella spp
Actinomyces naeslundii	Enterobacter spp
Lactobacillus casei	Mycobacterium fortuitum
Fusobacterium spp.,	Mycobacterium bovis (BCG strain)
Neisseria subflava	Mycobacterium marinum
Pichia pastoris	Salmonella enteritidis
Corynebacterium spp	Salmonella typhimurium

Conclusion

This change was not deemed significant by the GMSC as the additional agents to be used do not affect the overall risk of the contained use as the various routes of transmission of the new agents are no different to those already seen with the existing agents and the measures already in place were sufficient to control exposure to the proposed new agents. However, it was noted that the work was moving in a direction that might, at a later date, require a significant change notification to be made. Any further amendments made to the local assessment would be closely checked to ensure that these did not impact the original notification significantly.



Example 6: Modulation of gene expression in stem cells and the use of lentiviral vector systems in mammalian cell lines

Class 2 contained use

Outline of the Work

The project involved the generation of mammalian cell lines containing a variety of gene modulators which may have oncogenic properties. The original assessment identified that the genes may have oncogenic properties but specifically precluded any attempt to over express oncogenes. It also stated that sharps would not be used.

The contained use was notified around the time there was increased concern about use of lentiviral vector (LVV) systems that contained the posttranscriptional regulatory element of woodchuck hepatitis virus (the WPRE sequence) and so a decision was made to notify a significant change to this contained use so as to broaden it to cover all Class 2 projects using LVV systems in mammalian cell lines.

Proposed changes to GM contained use

Subsequent to the first change, further changes were proposed to extend the contained use to carry *out in vivo* work involving use of sharps and also to overexpress oncogenes.

Conclusion

The proposed changes were deemed significant as the use of sharps, together with the over-expression of oncogenes, required different control measures to those specified on the original notification.



Example 7: Genetic modification of *Leishmania* and *Trypanosoma brucei* species

Class 3 contained use

Outline of the Work

The aim of this work is to:

- introduce recombinant plasmid DNAs (containing *Leishmania*, *T. brucei brucei*, *T. brucei rhodesiense* and bacterial sequences) into *T. brucei* or non-infective *Leishmania* stages by nucleofection
- target specific genes for disruption (by homologous recombination) to generate null, loss-of-function or gain-of-function mutants, by the introduction of antibiotic resistance genes into chromosomal sites in the genomes of *T. brucei* or non-infective *Leishmania* stages.

The transgenic *Leishmania* and *T. brucei* produced characterised according to nucleic acid and protein content, viability and cell structure, infectivity in cultured macrophages, mammalian hosts, and whole animals. Transgenesis will be confined mainly to manipulations within genera i.e. insertion of *Leishmania* genes into *Leishmania* species; insertion of *T. brucei* genes into *T. brucei* species. There will be no inter-genera transgenic experiments.

Proposed changes to GM contained use

The proposed changes to the work were to:

- include the use of *Leishmania* (Hazard Group 2 and 3 species) infected sandflies; and
- carry out certain downstream sample analysis (includes cell sorting, cell imaging and fluorescent measurement techniques) at CL2 albeit supplemented by working practices routinely applied for handling *Leishmania* and *Trypanosoma* samples at CL3.

Conclusion

Following review by the GMSC Committee, it was agreed that the revised contained use using sandflies would increase the risk of exposure to parasites. Additional control measures would be required, including modification to the CL3 animal holding room and application of specific working practices to contain infected sandflies. Derogation from application of full CL3 measures for lower risk sample processing activities would mean a change in the conditions specified in the original consent from HSE.



Example 8: *In vitro* transfection of primary cells and cell lines for *in vitro* experiments to assess suitability for *in vivo* transplantation studies

Class 2 contained use

Outline of the Work

The overall goal of the research is to improve the viability, integration and therapeutic benefits of cells used for transplantation into the failing heart. This will be achieved by engineering cells to express a variety of either structural proteins which will enable improved connections and communication between donor and host cells or secreted proteins which will reduce cell death, aid myocardial regeneration and improve vascularisation. Reducing the expression of detrimental proteins will also be carried out. Candidate genes of interest or shRNAs for these genes (structural genes, growth factors, inflammatory pathway genes, cardiac transcription factors, oncogenes such as mTOR etc) will be over expressed in primary cells or established cell lines for *in vitro* co-culture experiments, or in the case of primary cells, for *in vivo* transplantation studies. Full characterisation (mRNA and protein expression levels, distribution of expression and cell growth characteristics) of the cells will be carried out prior to experiments commencing.

Genes or shRNAs will be delivered using a lentiviral vector system. Once cells have been infected and shown to be clear of virus particles, they will be used for *in vitro* experiments or for *in vivo* transplantation studies. The *in vivo* aspect of the project is Class 1.

Proposed changes to GM contained use

The amendment proposed was to the use of an adenoviral vector and additional genes to be expressed (including known oncogenes) using the new adenoviral or the existing lentiviral vectors

Conclusion

It was considered that the introduction of a new viral vector system, with a different route of exposure, along with introducing more hazardous oncogenic inserts for use in both vector types, presented both a different risk (aerosol route of exposure) and different consequences in terms of possible operator exposure (potential oncogenesis).

Conclusions

10. The ISTR BSG hope that the examples provided in this guidance help the biosafety community when they assess changes made to GM risk assessments; we would welcome any further examples from the community to keep the guidance as current and representative as possible.

11. This document deals with significant change but the BSG notes that many projects evolve over time with multiple minor changes made. Therefore there needs to be robust systems in place to identify when a project may have gone beyond what was originally notified. This could include setting boundaries in the original assessment/notification and identification of the most hazardous GMM that will be constructed. This should then provide clear reference points which can be used during review of any changes.

12. Finally, when making the decision as to whether a change is significant or not, consider what you would do if you were asked to submit the locally amended GM risk assessment to HSE in advance of an inspection. Would the amendments come as a surprise when compared to the work that had been originally notified or do they simply reflect a logical progression of the work?

Sources of further information

The Genetically Modified Organisms (Contained Use) Regulations 2014 – Guidance on regulations (L29) - <http://www.hse.gov.uk/pubns/priced/l29-2014.pdf>

The Scientific Advisory Committee on Genetic Modification Compendium of Guidance - <http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/index.htm>

ISTR and ISTR BSG - <http://www.istr.org.uk/> and <http://www.istr.org.uk/biosteeringgroup.shtml>