The Genetically Modified Organisms (Contained Use) Regulations 2014

Contained Use Notification

Notification of intention to conduct individual contained uses

- **If the contained use involves working with a biological agent that is listed on schedule 5 of the Anti-terrorism, Crimes and Security Act 2001, this form should not be e-mailed to HSE because it contains sensitive information. Please print the completed form and send it by post to the Notification Officer, Health & Safety Executive, Building 5S2 Redgrave Court, Merton Road, Bootle, Merseyside L20 7HS. The Schedule 5 list can be found at www.opsi.gov.uk/acts/acts2001/ukpga_20010024_en_18#sch5.**
- The public register sections MUST be understandable without reference to the risk assessment or other supporting documents.
- Please return your completed form to the Health and Safety Executive at the address given in Notes for Guidance.
- Please do not feel constrained by the box sizes - expand them or continue on separate sheets if necessary.
- Important - please refer to Notes for Guidance where identified.
- Fields marked with an asterisk (*) must be completed before the form is submitted to HSE.

FOR HSE USE ONLY

GM centre reference: GM	Date notification acknowledged:	Date contained use ceased:

Dates on which additional information submitted								
Date on which accident notification submitted								

☐ **Consent granted (class 3/4)** *(to be completed by HSE)*

Public Register

1. Organisation Details

* Name of organisation ***(note 1)***	University of Dundee
* Address	Main Campus, University of Dundee, Perth Road, Dundee, DD1 4HN
* Telephone Number	01382 344000
Fax Number	01382 201604
Email Address	university@dundee.ac.uk

Address(es) of premises where the contained use will actually be conducted (if different from that at Section 1) ***(note 1a)***

Public Register

2. Date of premises notification *(note 2)*

7/7/1979

* HSE Centre Number

GM 6

3. ☐ **Please check if notifying a connected programme of work *(note 3)***

4. * Class(es) of contained use - check all relevant boxes *(note 4)*

☒ Class 2 ☐ Class 3 ☐ Class 4 ☐ Contained use involving notifiable larger GMOs

☐ For class 3 or 4, check box if GMOs are likely to be imported from or exported outside the EC

Public Register

5. * Please give a short descriptive title of the contained use (or contained uses)

Use of genetic tools to study the basic biology/biochemistry of the Cryptosporidium parvum parasite, and to identify and validate potential drug candidates for cryptosporidiosis.

Public Register

6. * Purpose of the contained use *(note 5)*

To further scientific understanding of the basic biology/biochemistry of this human and animal parasite and to investigate potential therapeutic agents that could be used in a clinical setting.

Public Register

7. * Characteristics of the GMO(s) including the evaluation of foreseeable effects *(note 6)*

Recipient or parental organism

The intracellular parasite Cryptosporidium parvum will be used as a model organism due to the fact that out of 32 identified species of Cryptospordium it is one of only two species that cause disease in humans. Further, this species is amenable to genetic manipulation and is easily maintained in the laboratory. The effects of clinical infection with Cryptosporidum parvum are well known and range from asymptomatic to severe and sometimes fatal illness. However, the latter is only seen in immunocompromised indviduals or those with severe underlying health issues. Most people who contract cryptosporidiosis make a full recovery without treatment.

Public Register

* Host / vector system

The host is C. parvum Iowa II strain. This is the only strain of Cryptosporidium that will be used. To generate GMO Cryptosporidium, the organism is transfected with (1) A CRISPR/Cas9 plasmid containing a guide RNA sequence that targets the gene of interest and (2) a linear DNA fragment that contains a gene for NanoLuciferase and a selection marker. The plasmid speificially contains Streptococcus pyogenes Cas9 gene under control of Cryptosporidium regulatory elements. This plasmid also contains the Cryptosporidium U6 promoter to produce a guide RNA. The resulting RNA molecule directs the activity of Cas9 to act against a specific sequence of the Cryptosporidium genome. Plasmids are not maintained in Cryptosporidium, therefore Cas9 and the guide RNA are only transiently expressed by Cryptosporidium and are not maintained from generation to generation. However, the linear DNA molecule containing the gene for NanoLuciferase and a selection marker (neomycin resistance gene) is incorporated into the genome at the site designated by the guide RNA. The guide RNA "guides" Cas9 to make a double stranded break in the chromosomal DNA at a specific sequence and the endogenous homologous repair machinery of Cryptsporidium repair this break by integrating the linear DNA fragment. This linear DNA fragment modifies the chromosomal DNA, for example by deleting a gene of interest, or by inserting an epitope tag at the C-terminus of the encoded protein (to allow for expression and localization studies). To encourage homologous recombination at the site designated by Cas9, the linear DNA fragment is flanked with regions homologus to the chromosomal insertion site. This integrated DNA fragment will be inherited by all progeny organisms and maintained in the chromosomal DNA.

Public Register

* Origins and intended functions of the genetic material involved

The genetic material is either C. parvum genetic material being used to generate mutant strains or it is marker genes (GFP, YFP, etc) to identify expression patterns, reporter genes (luciferase, etc) to study expression levels, epitope tags (HA,GST, etc) for protein purification or the drug resistance marker (Neomycin resistance) that is needed to select for transgenic organisms. All these materials will be derived synthetically or via production of genetic material in E. coli using standard molecular biology approaches.

7. Characteristics of the GMO(s) including the evaluation of foreseeable effects *(cont'd)*

* Evaluation of foreseeable effects

It is foreseeable that workers could become infected with C. parvum, either the parental strain (Iowa II) or any of the genetically modified strains. It is considered that none of the mutations are likely to increase pathogenicity of the organism and, indeed, many of the mutations will likely be deleterious to the organism. Neomycin Resistance (confers resistance to paromomycin) is introduced into the GM organisms in order to select for the genetic change. Paromomycin is an effective drug for treatment of cryptosporidiosis in immunocompromised mice, but not in human cryptosporidiosis and so will not affect the virulence of the organism.

The inherent homologous recombination machinery of C. parvum will be used to integrate transgenes at specific locations in the genome. All transgenes used to generate transgenic Cryptosporidium strains are chromosome borne. Currently there is only a single drug resistance marker for Cryptosporidium, therefore only a single cassette of transgenes may be introduced into a strain. Transgenic C. parvum are maintained under drug pressure, selecting for growth of only transgenic organisms. Additionally, all transgenic strains are produced from the same parental C. parvum Iowa II strain, so recombination or gene transfer from transgenics to Wild Type does not increase the genetic diversity. Cryptosporidium do not stably maintain plasmids, therefore transgenes are all chromosome borne. Transgenes expressed by Cryptosporidium parvum are not transferred from Cryptosporidium to its host (human cells in co-culture or infected animals - humans included).

Cryptosporidium are water borne, but cannot replicate in the environment. Cryptosporidium parvum can only survive and grow inside the host organism (cow, sheep, humans, immunocompromised mice).

Public Register

8. Containment and control measures for larger GMOs (eg GM animals and plants) *(note 7)*

Public Register

Contained Use Notification

9. Maximum culture volumes per experiment - for GMMs only *(note 8)*

(i) Class 2 contained use, state approximate culture volume

C. parvum cannot be cultured continously in vitro, so there are no culture volumes. However, it is estimated that there will be a maximum volume of 500ml infected feacal material being stored in aliquots no bigger than 50ml at any one time.

Public Register

(ii) for class 3 or class 4 contained use, specify the culture volume

Public Register

10. For GMMs only, indicate the level of containment that will be applied (please check the appropriate box(es)) *(note 9)*

	Level 2	Level 3	Level 4
Laboratory contained use	☒	☐	☐
Glasshouses	☐	☐	☐
Growthrooms	☐	☐	☐
Animal Units	☒	☐	☐

Public Register

Large scale contained use (ie contained use to which Table 2, Schedule 8 containment is appropriate	☐	☐	☐
Human clinical applications	☐	☐	☐

11. For GMMs only - application for any derogation from full containment for the class of contained use (measures and justification) *(note 10)*

12. * Describe the waste management measures which you will apply to the contained use (including the type and form, treatment, degree of kill, proposed process testing / monitoring measures, ultimate form and fate) *(note 11)*

Water bottles and used feed must be autoclaved prior to washing or disposal by placing in taped closed - autoclavable biohazard bag. Autoclave using SLS standard conditions. Dirty bedding and isolator units must be autoclaved prior to disposal by placing in taped closed, autoclavable biohazard bag - autoclave using SLS standard conditions. Flasks, pipettes, plates, glassware, spent media, etc will be autoclaved prior to washing or disposal by placing in taped closed, autoclavable biohazard bag - autoclave using SLS standard conditions. Spills/waste (media, feces, tissue, towels, etc) from sample collection will be placed into a biohazard bag in a biohazard bin; other surface spills will be treated with 3-6% H2O2 application for 20 minutes. Any needles/blades used for work or unintentional sharps (from broken glass/plastics) will be placed in a sharps container which is then disposed of via standard SLS clinical sharps disposal route. Biosafety cabinet/work areas - 3-6% H2O2 application for 20 minutes, or use of Diversey Oxivir Disinfectant Spray (or similar) and wipe down following handling of parasites on surfaces or nonporous materials contaminated with parasites. H2O2 made fresh every 30 days.

13. * Is an emergency plan required according to regulation 21?

☐ Yes ☒ No

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Contained Use Notification

Public Register

Public Register

☐ If 'Yes', please check to confirm that it is attached to this form

14. ☒ *** Please check to confirm that you have attached a risk assessment to this form** ***(note 12)***

☐ Please check if you are claiming exemption from disclosure for sections of the risk assessment

Public Register

15. * Please enter comments of the genetic modification safety committee on the risk assessment ***(note 13)***

Dear all,

Just to add that Neopredisan, a chlorinated phenol derivative, is a commonly used disinfectant against Cryptosporidium and other endoparasites in laboratory animal facilities and in agriculture, at least in Europe. It is pretty fearsome stuff, but might be an alternative to hydrogen peroxide in some instances (for example in the final decontamination of items prior to their disposal).

Best wishes

LN

Hi Mattie,

I said I would reply regarding your RA document. You have done a very good job with it and so I have no real issues. Just some comments and a couple of questions.

Overview

You make a comment about greater risk for the young and immunocompromised. This means that there should be extra consideration given to those working with the organism who are immunocompromised or who have young children. Especially, in this regard to pregnant persons and those who have recently given birth/breast feeding. The decision will be yours, but you may wish to ensure that any such persons are given the option not to work with the organism. Also, anyone else who is significantly immunocompromised (they of course must disclose this to you first).

2a – hazards to human health. I like that you have commented about using an attenuated strain if this becomes available. It shows you have considered this (even though at present such a strain is not available). Likewise the point about in vitro culture (although that is not directly related to human health).

Section 3 – I agree that the provisional containment level is 2. You make a comment about it not being able to be aerosolised. What do you mean by this? I am sure it could be made into an aerosol, but the desiccation that would then occur would kill it. Is that what you mean?

4a – direct effects on the environment of the host. It is endemic and so there are no issues. That is all that needs to be said.

4b. The genetic material. You do mention about the antibiotic resistance cassette used being effective against an antibiotic that is relevant to treatment in immunocompromised mice. Does this have any relevance to wild mice or other organisms in the wider environment?

5b. You answered no, but I would like more clarification about the aerosol issue.

Spill process. Do you have a special spill procedure for uncontained spills? Eg.stuff gets on the floor of your faecal room; what do you do? Is H2O2 wash down of floor needed?

NH

Public Register

16. Personal Information

* Name of person responsible for supervision and safety of GM contained use at the premises

Professor J.J. Blow FRSE FMedSci (Dean of the School of Life Sciences

Training and qualifications

BSc (Hons) first class, Pathology, University of Edinburgh, 1984
PhD in Zoology, University of Cambridge, 1987

NON DISCLOSURE OF INFORMATION

17. Enter in this section any information required in sections 1-15 which you do not wish disclosed, together with full justification *(note 14)*

18. Declaration

I am notifying an intention to carry out contained use of genetically modified organisms with the authority and approval of the person (organisation or individual) named in section 1 of this form

Name	Dr Nicholas R Helps BSc, PhD, CMIOSH, MISTR		
Position in	Health and Safety Advisor, School of Life Sciences		
Signed *(note 15)*		Date	25/08/2017

THE GENETICALLY MODIFIED ORGANISMS (CONTAINED USE) REGULATIONS 2014
NOTIFICATION OF INTENTION TO CONDUCT INDIVIDUAL CONTAINED USES

NOTES FOR GUIDANCE

Data Protection Act 1998

This Act requires the Health and Safety Executive (HSE) to inform you that this form may include information about you (this is called "personal data" in the Act) and that we are a "data controller" for the purposes of the Act. HSE will process the data for health, safety and environmental purposes. HSE may disclose these data to any person or organisation for the purposes for which it was collected or where the Act allows disclosure. As data subject, you have the right to ask for a copy of the data and to ask for any inaccurate data to be corrected.

All the information given in sections 1-15 of this form will be placed on HSE's public register of notifications within 14 days of receipt. You may consider that there is information relevant to these sections whose disclosure would adversely affect your organisation's competitive position, intellectual property rights or which you do not wish disclosed on other grounds referred to in the Environmental Information Regulations (EIR) 2004, regulation 12. If so, you should enter such information in section 17 with a full justification for its exemption from disclosure. However, it should always be possible to provide some information in these sections for the public register. The Competent Authority will decide whether the information in section 17 will be exempt from disclosure and will notify you of its decision in writing.

Personal information will not be disclosed unless the individual concerned has given his or her explicit written permission.

Compliance with other legislation

It is important to note that compliance with the provisions of the Contained Use Regulations does not constitute compliance with other relevant legislation. For example, you may also need to apply separately for licences or permits under legislation controlling plant health, animal scientific procedures, or the introduction of non-indigenous species. For clinical trials involving gene therapy, you will need ethical approval.

Even if you have fulfilled the requirements of the Contained Use Regulations, and have any necessary consents or approvals under that legislation, you **cannot begin** the contained use unless you also have the relevant licences / permits under any other applicable legislation.

Note 1

This will normally be the University, Institution, Company or Organisation. Only rarely will it be necessary to include an individual's name.

Note 1a

If you intend to carry out contained use involving GMMs, you must not leave this section blank unless you are claiming exemption from disclosure. If you are claiming that the precise address of the premises where contained use with larger GMOs (eg GM animals or plants) are to be carried out should not be disclosed, you must include this, together with the justification, in section 17.

Note 2

If you have previously notified your premises, indicate the date of the notification and the HSE reference number assigned (eg GM111). **If you have not notified your premises**, you will no have a reference number so please contact the notification officer (see note 15) or email **bioagents@hse.gsi.gov.uk** for a GM centre reference number. Note that if not previously notified, you will also have to complete a premises application notification - and submit it at the same time as this contained use notification. The fee payable in such cases will only be that related to the contained use notification.

Note 3

It is permissible to notify a connected programme of work using this form. However, you must include details of all of the component contained uses in sections 4-15. The fee payable in relation to connected programmes is the fee for the highest class of contained use involved. (notifiable contained use involving larger GMOs are equivalent to class 2 for this purpose).

Note 4

Please check all applicable boxes. For class 3 and 4: The EC Regulation 1946/2003 on transboundary movements of Genetically Modified Organisms requires Member States to inform the Biological Clearing House and the European Commission of any decisions on class 3 and class 4 contained use involving GMMs that are likely to be subject to transboundary movements. Transboundary movements are those entering or leaving the European Union. In order for this information to be collected, please

check the box if your class is 3 or 4 GMMs are likely to be subject to such transboundary movements. Any information you do not wish disclosed should be entered in section 17 together with the justification.

Note 5

Any information you do not wish disclosed should be entered in section 17 together with full justification.

Note 6

For contained use involving GMMs, this section **cannot** be left blank unless you have a justified request for non-disclosure in respect of protection of intellectual property rights. If you are not making a request for non-disclosure in respect of intellectual property rights, you must at least include general characteristics of the GMMs involved in the intended contained use. Where there are no justifiable requests for non-disclosure, you must include precise details. An evaluation of the foreseeable effects must also be included, in as precise detail as possible. The evaluation of foreseeable effects should include the identity and characteristics of the GMMs indicated by the risk assessment. Include information on hazards to human health and the environment with particular reference to those arising from the modification as opposed to being inherent properties of the host micro-organism (a fuller account of these details will be included in the risk assessment).

For contained use involving larger GMOs, it is permissible to request non-disclosure for any of the required information, but the second section should still be completed in as precise detail as possible taking into account it may be disclosed. The evaluation of foreseeable effects is required to consider only human health and safety aspects. Any information you do not wish disclosed should be entered in section 17 together with the justification.

Note 7

For contained use involving larger GMOs, describe the containment and control measures which you will apply to the contained use. These should be justified by reference to the risk assessment. Any information you do not wish disclosed should be entered in section 17 together with the justification.

Note 8

Any information you do not wish disclosed should be entered in section 17 together with the justification.

Note 9

You must not leave this section blank.

Note 10

For contained use involving GMMs, you will normally need to apply all the measures specified as requirements for the relevant containment level. If, however, your risk assessment indicates that any of those measures are unnecessary, you may ask for permission to omit them by requesting a derogation(s). Indicate any such measures with a brief justification for the derogation that includes references to the relevant parts of the risk assessment. You **cannot** request non-disclosure for the actual containment measures (unless your intellectual property rights might be affected) BUT you may wish to request exemption for the justification. If a request is made for non-disclosure, the exempt information must be included in section 17 together with the justification.

Note 11

Waste management measures which will be applied to the contained use must be described. You should take into consideration only the waste consisting of or containing viable GM material. You must specify the type and form of waste(s) generated, their treatment and proposals for testing / monitoring the inactivation process. For contained use involving GMMs, this section cannot be left blank unless you are claiming protection for reasons of intellectual property rights. Even if this is not the case, it is permissible not to give precise details if claims for non-disclosure can be justified. For instance, you could say that inactivation is by heat treatment to give 100% kill, but the precise detail of how this is achieved may be commercially confidential information. If a request is made for non-disclosure, the information must be included in section 17 together with the justification.

Note 12

You must attach the risk assessment of the contained use to this form. The risk assessment will not be placed on the public register, but will be open to disclosure to members of the public on request (subject to exemption provisions).

If you wish to claim exemption from disclosure for any sections of the risk assessment, please indicate those sections clearly on the risk assessment and set out a full justification for exemption. If a request for information is received and your justification for non-disclosure is accepted, the risk assessment will be disclosed with the exempt sections removed. You are advised to submit a second version of the risk assessment from which those sections have already been removed. If it is decided, in the public's interest, to release the information, you will be informed of this decision in writing.

Note 13

NB Remember that, as well as consulting the genetic modification safety committee on the risk assessment, you must also comply with the Safety Representatives and Safety Committees Regulations 1977 and, where any employees are not in groups covered by trade union safety representatives, you must consult such employees according to the Health and Safety (Consultation with Employees) Regulations 1996. If you do not wish some of the information to be disclosed, the exempt information must be included in section 17 together with the justification.

Note 14

Please enter in this section any information, required in sections 1-15, which you wish to be exempt from public disclosure on grounds that:

(a) disclosure would harm your organisation's competitive position;
(b) disclosure would compromise your intellectual property rights; or
(c) the information falls into one of the other categories for exemption in the regulation 12 of EIR 2004, state which.

For each piece of information entered you must:

(a) state clearly which the grounds applies. In particular, state which category of exemption allowed by the EIR 2004 applies, namely disclosure would adversely effect:

- international relations, defence, national security or public safety
- the course of justice
- confidentiality of proceedings
- commercial / industrial confidentiality
- intellectual property
- protection of the environment

(b) indicate the section of the form to which it is relevant; and

(c) provide a full justification, explaining why the stated ground for exemption applies

You do not need to enter any personal information as this information is covered by the Data Protection Act and will automatically be treated as confidential.

Note 15

Send the completed form by email to:

bioagents@hse.gsi.gov.uk

Or alternatively by post to the address below:

Notifications Officer
Health and Safety Executive
Building 5S2, Redgrave Court
Merton Road
Bootle
Merseyside
L20 7HS

Note 16

If the contained use involves working with a biological agent that is listed on schedule 5 of the Anti-terrorism, Crimes and Security Act 2001, this form should not be e-mailed to HSE because it contains sensitive information. Please print the completed form and send it by post to the Notification Officer, Health & Safety Executive, Building 5S2 Redgrave Court, Merton Road, Bootle, Merseyside L20 7HS. The Schedule 5 list can be found at www.opsi.gov.uk/acts/acts2001/ukpga_20010024_en_18#sch5.