



**ADVISORY COMMITTEE ON RELEASES TO THE ENVIRONMENT**

***Advice on an application for Deliberate Release of a GMO during a Clinical Trial***

**Applicant:** Microscience Ltd

**Application:** To release genetically modified live deletion attenuated *Salmonella typhi* as part of a human clinical trial to test the effectiveness of a vaccine against travellers' diarrhoea.

**Ref:** 02/R37/01<sup>1</sup>

**Date:** 28<sup>th</sup> February 2003

**Advice of the Advisory Committee on Releases to the Environment to the Secretary of State under section 124 of the Environmental Protection Act 1990**

ACRE is satisfied that all appropriate measures have been taken to avoid adverse effects on human health and the environment from the proposed release and sees no reason for the release not to proceed on the following condition.

The holder of the consent shall notify the following information at the times shown:

The effects of the release as authorised by the consent, for the assessment of any risks there are of damage to human health and the environment from the GMOs concerned. This should be in the form of a report submitted:

- by one month after the date of administration of the GMO; to evaluate shedding of the GM bacteria during the clinical trial by assessment of the quantity and period over which shedding of the GMO from volunteers occurred. This to be provided with a report on the general health of the volunteers.

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<sup>1</sup>Application reference 02/R37/01 dated 13<sup>th</sup> June 2002 taking into account all information and amendments as in the applicant's letters dated 24<sup>th</sup> July 2002, 19<sup>th</sup> August 2002, 9<sup>th</sup> October 2002, 1<sup>st</sup> November 2002, 7<sup>th</sup> January 2003, 21<sup>st</sup> January 2003, 6<sup>th</sup> February 2003, 17<sup>th</sup> February 2003 and 24<sup>th</sup> February 2003

## Background

Naturally virulent enterotoxigenic *Escherichia coli* (ETEC) is a common cause of dehydrating diarrhoea worldwide. A significant proportion of travellers returning to the UK from overseas excrete ETEC in their stools<sup>2</sup>. Such individuals constitute a source of continuous release of natural virulent ETEC into our sewage system.

### *The GM strain*

Microscience Ltd developed the trial GM vaccine using a strain of *Salmonella typhi* in which a gene involved in metabolism and another gene for growth and survival have been mutated so that they are no longer functional. These mutations reduce the pathogenicity of the organism and its ability to survive, multiply and cause disease. The attenuated strain then underwent a second genetic modification in which a gene for a non-toxic antigen from enterotoxigenic *Escherichia coli* (ETEC) was inserted. The GMO will be used as a vaccine to deliver the ETEC antigen to cells where an immune response would be stimulated.

ACRE requested further information on the rationale behind use of *Salmonella typhi* as the vector. *S. typhi* was chosen as the vector for this vaccine because of its restricted host range (humans are the only natural reservoir) and because it will elicit the right kind of immune response against the ETEC antigen while being destroyed in the process. Conventional *Salmonella typhi* has been used clinically as a background strain for constructing candidate typhoid vaccines and forms the basis for the current live (non-GM) oral typhoid vaccine.

### *The clinical trial*

To test the efficacy of the vaccine, the proposal is to give the vaccine orally to healthy volunteers at St George's Hospital, London. The consenting volunteers will be able to leave 48 hours after the vaccination to go about their normal daily activities. Microscience expect that some of the participants may excrete viable GM bacteria in their stools for up to 7 days following vaccination and this will pass into the sewage system. Travel will be restricted due to the requirement to attend the hospital daily and the volunteers are not permitted to leave England until they are no longer excreting GM bacteria. In the event that a subject is considered to have a clinically-significant infection with the GMO they will be treated with antibiotics and followed up until the infection is clear. The infection will only be considered clear if blood, urine and stool samples are all clear on two consecutive days.

### *The Assessment Process*

The application was submitted by Microscience on 13 June 2002. It was discussed by ACRE at three meetings on 4<sup>th</sup> July 2002, 5<sup>th</sup> September 2002 and 23<sup>rd</sup> January 2003. Minutes of these meetings can be viewed on the ACRE website<sup>3</sup>. The Director of the Laboratory of Enteric Pathogens at the Central Public Health Laboratory was co-opted onto the committee to strengthen our expertise in assessing this proposal.

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<sup>2</sup> J Clinical Microbiology 38 (10) :3550-4

<sup>3</sup> <http://defraweb/environment/acre/index.htm>

## Comment

In coming to its conclusion ACRE considered not only the risk to the environment and to people in the wider community, but also considered the safety of volunteers in the trial. In arriving at its advice Members considered the application against the requirements of the legislation and in particular:

- What is the likelihood of and consequences for reversion of the GM vaccine strains to the wild-type state?
- Is the host range of the strains altered? Could they infect livestock?
- Will these strains persist in the environment as self-sustaining colonies?
- Will they harm the environment?
- Can the strains be detected and identified in the environment – particularly sewage where most will be shed which is replete with similar bacteria?
- Are the volunteers who ingest the vaccine safe? Will those in the wider community be safe?

The following section briefly sets out the committee's advice on each of these issues:

*What is the likelihood and consequences of reversion to the wild-type bacterium?*

In making an assessment on the likelihood of this happening, the committee requested additional data on the attenuating mutations that have been introduced into these strains and on the similarity with homologous genes from other bacterial species. The Committee also requested that Microscience review their application to include a full risk assessment of the potential risk arising from reversion of the GMO to wild type.

Reviewing the data, the Committee was satisfied that the mutations were stable and that the likelihood of reversion to wild type by acquisition of wild type genes was very low. In particular the Committee took into account that under natural conditions it is considered unlikely that *Salmonella* are able to receive genetic material from other organisms because *S. typhi* demonstrates little genetic variation worldwide, appearing almost as clones. In the event that the GMO did acquire a wild type gene B it is unlikely to replace the normal function since gene B shows only low levels of homology between species and functional complementation is unlikely to occur. It was also considered highly unlikely that the GMO would acquire a replacement for both mutated genes.

*Is the host range of the strain altered?*

*S. typhi* is very host specific. There are no known animals, plants or insects that it can colonise or use as a vector for transmission. The genetic modifications in the vaccine strain have been designed to reduce growth and survival of the GMO and not to change the host range. Attenuation of the GMO makes it weaker than the wild type strain and puts it at a competitive disadvantage. It is therefore not likely to be more competitive as regards host range and pathogenicity. ACRE was satisfied that the host range for the GM bacterium is restricted to humans and that this had not been changed as a result of the modification.

*Will these strains proliferate in the environment as self-sustaining colonies?*

ACRE noted the considerable number of inaccurate statements made in the application concerning treatment of sewage and monitoring of water quality and the poor level of general understanding of the interaction between these systems, particularly in respect of the risk assessment for the proposed release. ACRE requested that Microscience provide a better consideration with regard to these aspects of the application. ACRE noted Microscience's reliance upon chlorine in water to inactivate any GMO shed from volunteers and also monitoring of coliform counts. Chlorinated water may not be available or chlorine may be inactivated by organic matter. Monitoring of coliform counts is performed to ensure that drinking water is not contaminated and would not contribute to monitoring of levels of the GMO shed into the sewage system. ACRE requested that Microscience reconsider their application and particularly their risk assessment to reflect more accurately the actual situation.

ACRE were satisfied with the applicant's response. ACRE also considered that the nature of the organism and the attenuating mutations could be relied upon to prevent the multiplication of the GM strain in humans and the environment and that the GMO is severely attenuated. Transmission of wild type *S. typhi* depends upon direct transmission, it does not persist in the environment and there is no animal reservoir. ACRE is satisfied that the likelihood of proliferation and persistence in the environment is very low.

*Will the GM vaccine strains harm the environment?*

*S. typhi* is very host specific. There are no known animals plants or insects that it can colonise or use as a vector for transmission, it does not persist in the environment and the GMO is disabled so that it cannot multiply. ACRE was also satisfied that the likelihood of the GM strain obtaining a double reversion of the attenuating mutations was low. ACRE considered that the risk to the environment was very low.

*Can the strains be detected and identified in the environment?*

Using the detection methods specified, ACRE was satisfied that it is possible to discriminate the GM strains from the general bacterial population. Of relevance to the Committee was whether the sensitivity of the procedures were adequate to monitor the volunteers and any potential identified hazard. In this respect the Committee requested further information on the media to be used to culture the GMO for detection purposes. The Committee was satisfied that the sensitivity of the method was satisfactory. The Committee requested information on monitoring of the volunteers and was satisfied with the proposed plan, requesting that a report of this monitoring be provided after the release to contribute to the ongoing process of risk assessment and evaluation of level of shedding of the GMO.

*Are the volunteers who ingest the vaccine safe? Will those in the wider community be safe?*

In coming to a view on this the ACRE reviewed the applicants risk assessment, plans for monitoring of the volunteers and the emergency plans. The Committee requested

that Microscience give further consideration in their risk assessment of the potential hazard towards contacts of the volunteers including pregnant individuals and the immunocompromised. The Committee was satisfied with the response and that the design of the trial was based on established good clinical practice guidelines. ACRE noted the approval of the Medicines Control Agency (MCA), whose primary duties are to ensure safety, health and well-being of all participants in these studies. The Protocol for testing of this new vaccine strain includes a dose-escalation phase, to further reduce the risk of adverse effects on volunteers and the protocol provides for an early treatment with ciprofloxacin (to which the vaccine strain is sensitive) in the event of a clinically-significant infection with the GMO developing or of evidence of a reaction to the vaccine.

ACRE concluded that the risk to the wider population was very low largely because exposure to levels of bacteria capable of infecting people is remote. The Committee notes that as for any other clinical trial, this trial is subject to other approvals.

#### *Items arising from Public Representations*

ACRE considered the two representations received from Members of the public in respect of any science issues which might impact on this application. They noted and considered comments from the public which are summarised briefly here:

- There is a possibility of volunteers not remaining in London
- shedding of the GMO may occur away from the normal sanitation facilities or that normal sewage treatment or chlorinated water will not be available.
- unmodified *S typhi* is a human pathogen
- potential for reversion to wild type by acquisition of the attenuated genes and recombination
- potential transmission of the GMO to contacts of the volunteers
- potential risk to volunteers
- excretion of the GMO could extend beyond 7 days

ACRE was content that all science-based issues raised in the representations had been considered thoroughly during the Committee's assessment of the dossier and that no outstanding issues remained. ACRE was satisfied that no new issues had been raised.