Department of the Environment, Food and Rural Affairs

Advisory Committee on Releases to the Environment Format 2: Release of genetically modified organisms other than higher plants

PART C: SUMMARY NOTIFICATION INFORMATION FORMAT (SNIF) FOR RELEASES OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS

A. General information

1. Details of notification

Member state of notification.	United Kingdom
Notification number.	B/GB/03/R35/02
Date of acknowledgement of notification.	3 January 2003
Title of the project.	Phase I clinical trial of a live attenuated *Salmonella typhi* vaccine for the prevention of typhoid fever
Proposed period of release.	Six-month period between April 2003 and April 2005

2. Notifier

Name of institution or company. Acambis Research Ltd, Peterhouse Technology Park 100 Fulbourn Road, Cambridge, United Kingdom CB1 9PT

3. GMO characterisation

a. The GMO is a:

- bacterium

b. Identity of the GMO.

- Strain of *Salmonella typhi* from which the chromosomal genes *aroC*, *aroD* and *htrA* have been deleted.

c. Genetic stability

- High.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 5 (1))?

No.

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

No.

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notify?

No, although a strain of identical phenotype and genotype has been previously released in the US (no official notification of release).

7. Summary of the potential environmental impact of the release of the GMOs

This release application covers a genetically modified strain of *S. typhi* designed to be used as a live human vaccine for the prevention of typhoid fever. The strain has been attenuated by the introduction of large deletion mutations into chromosomal genes.

The vaccine strain has been derived by deleting functions from a naturally-occurring strain. No new genetic material has been introduced.

The potential environmental risks associated with the use of this strain as a vaccine relate to either the possible horizontal transfer of genetic information to or from other bacterial species that inhabit the same niche, namely the human GI tract or to the possibility that the attenuations introduced into the strain will not be sufficient to prevent typhoid-like symptoms.

The GMO is no more or less able to transfer or receive genes than normal commensal enterobacteria or wild-type *S. typhi*. *S. typhi* strains circulate widely in the world, simultaneously with large numbers of other bacterial enteropathogens (e.g. pathogenic *E. coli*, *Shigella* sp., *Campylobacter* sp., *Vibrio* sp. etc) but remain a readily recognisable bacterial species. This demonstrates that in nature horizontal gene transfer which is relevant to this assessment, namely transfer which would confer altered capacity to cause disease, is not observed to occur.

During this experimental release a controlled dose of the GMO will be administered to a small population of volunteers under closely supervised conditions in which the incidence of concurrent bacterial GI infections will be close to zero. It is therefore considered highly unlikely that such gene transfer could result in any recombinant strains more hazardous than the parental organisms involved in the transfer, or than the significant numbers of natural enteropathogens released into the sewers every day.

The likelihood of the GMO retaining functional ability to cause disease is very low, owing to the nature of the attenuations introduced. In addition, clinical trial data from 100 volunteers, using a vaccine with identical phenotype and genotype, revealed no serious adverse events attributable to the vaccine and an absence of bacteraemia (indicating a lack of invasive capability). Even if such a disease-causing capacity were to remain, this would be identified early in the trial following exposure of only a small number of individuals who could be effectively clinically managed with antibiotic therapy. The risk to the wider environment would be effectively zero as the GMO is effectively destroyed in the sewer system and cannot survive in many environments.

B. Information relating to the recipient or parental organisms from which the GMO is derived

1. Indicate whether the recipient or parental organism is a:

bacterium

2. Complete name

genus	Salmonella
species	enterica
subspecies	
strain	ACAM948-CVD
serovar	typhi
common name	*S. typhi*

3. Geographical distribution of the organism

a. Indigenous to, or otherwise established in, the United Kingdom?
No, but will rarely be brought into the UK by returning travellers from endemic regions.

b. Indigenous to, or otherwise established in, other EC countries?
No, but will rarely be brought into the EC by returning travellers from endemic regions.

If Yes, indicate the type of ecosystem in which it is found:
Only associated with human carriage.

c. Is it frequently used in the United Kingdom?
No.

d. Is frequently kept in the United Kingdom?
Yes.

4. Natural habitat of the organism

a. If the organism is a micro-organism:
water
soil, free-living
other: Wild-type *S. typhi* strains are human enteropathogens which are shed in faeces and contaminate water sources and foodstuffs via irrigation/fertilisation with contaminated material.

5. a. Detection techniques:
For detection of the GMO in environmental samples, part of the sample is first plated on SC-agar supplemented with “aro-mix”. The GMO itself is also plated separately onto this medium to serve as a reference. Colonies which grow from the environmental sample that have the appearance of the GMO on the reference plate are picked and spotted onto SC-agar and SC-agar supplemented with “aro-mix”. Most bacteria will then grow on both types of media whereas the GMO can grow only on the SC-agar supplemented with “aro-mix”.

b. Identification techniques:
Species can be confirmed as *S. typhi*, if required, by commercially available kits such as api20 or api50 (http://www.biomerieux.com/). The GMO can be identified absolutely specifically by phenotypic and genetic analyses, e.g. PCR.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes: Wild-type *Salmonella typhi* is classified as a Biological Agent in hazard group 3. The immediate parent of the GMO in this application has received exemption and is classified as a Group 1 recipient (or host). This strain has been additionally attenuated by a further deletion of the *htrA* gene to produce the GMO, ACAM948-CVD.

The parental strain, Ty2, is classified as Biosafety Level 2 by the American Type Culture Collection (ATCC).

7. Is the recipient organism pathogenic or harmful in any other way (including its extracellular products) either living or dead?

Yes: Like all other gram-negative organisms the outer membrane contains lipopolysaccharide, or endotoxin, which can be harmful if injected.

If Yes,

a. to which of the following organisms:
humans
animals

b. pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms:
The GMO is designed to be administered to humans as a vaccine to prevent typhoid fever. As such it is expected to be non-pathogenic for humans, as demonstrated in previous clinical trials of genetically similar strains. Wild-type *S.typhi* is only known to colonise and infect humans. The GMO carries no known prophage.

8. Information concerning reproduction

a. Generation time in natural ecosystems:
The GMO has been shown not to replicate under the environmental conditions to which it will be exposed.

b. Generation time in the ecosystems where the release will take place:
GMOs will reproduce in GI tracts of volunteers, generation time unknown.

c. Way of reproduction:
Asexual.

d. Factors affecting reproduction:
The organisms will colonise and reproduce in the human GI tract. They are expected to be transiently shed in the faeces over a period of up to 5 days. They are not able to reproduce in a variety of environmental conditions and therefore are not expected to multiply following shedding.

9. Survivability

a. Ability to form structures enhancing survival or dormancy:
None.

b. Relevant factors affecting survivability:
The organisms are deficient in the genes *aroC* and *aroD* which causes them to be auxotrophic for certain aromatic compounds not found in the environment.

10. a. Ways of dissemination:
Shed in faeces of recipients.

b. Factors affecting dissemination:
Volume of faeces and location of excretion. It is assumed that all will be properly excreted into a WC flushed with chlorinated water and collected into the sewer system for containment and treatment.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the United Kingdom (give notification numbers)

None.

C. Information relating to the genetic modification

1. Type of the genetic modification

Deletion of genetic material:
Deletion of chromosomal DNA from *aroC*, *aroD* and *htrA* genes.

2. Intended outcome of the genetic modification

A live attenuated strain which will form an oral vaccine to protect against typhoid fever.

3. a. Has a vector been used in the process of modification?
Yes.

b. If yes, is the vector wholly or partially present in the modified organism?
No.

If No, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information:

Not applicable.

5. If the answer to questions B 3 (a) and (b) is no, what was the method used to introduce the insert into the recipient / parental cell?

Not applicable.

6. Information on the insert

Not applicable.

D. Information on the organism(s) from which the insert is derived (Donor)

Not applicable as this GMO contains no inserted genetic material.

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

a. Is the GMO different from the parental organism as far as *survivability* is concerned?
Yes.
The deleted *aroC* and *aroD* genes makes the GMO auxotrohic for aromatic compounds and reduces its ability to replicate ex-vivo. The *htrA* mutation renders the GMO less resistant to killing by macrophages and renders the GMO unable to access the bloodstream after oral ingestion.

b. Is the GMO in any way different from the parental organism as far as mode and/or rate of *reproduction* is concerned?
No.

c. Is the GMO in any way different from the parental organism as far as *dissemination* is concerned?
No.

d. Is the GMO in any way different from the parental organism as far as *pathogenicity* is concerned?
Yes.
The decrease survivability outlined in 1a is anticipated to result in an inability to cause disease (i.e. typhoid fever) in human vaccine recipients.

2. Genetic stability of the genetically modified organism

High.

3. Is the GMO pathogenic or harmful in any other way (including its extracellular products), either living or dead?

No, but see below:

a. to which of the following organisms:
humans.

b. in relation to human health -

i. the toxic or allergenic effects of the non-viable organism and/or its metabolic products,
The LPS component of the outer membrane is toxic if injected.

ii. the product hazards,
None known if administered orally.

iii. the comparison of the organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity,
Three genes have been deleted from the chromosome of the organism, rendering it auxotrophic for aromatic metabolites and more readily killed by macrophages, to create the GMO which is designed as a vaccine.

iv. the capacity of the organism for colonisation,
The GMOs are expected to colonise the G.I. tract of volunteers who are fed them and be shed transiently for up to 5 days.

v. if the organism is pathogenic to humans who are immunocompetent, The GMO is designed as a vaccine to protect immunocompetent people from infection with their virulent, wild-type counterparts.

4. Description of identification and detection methods:

a. Techniques used to detect the GMO in the environment,
Standard plating methods are used to detect and identify *S. typhi* in the environment. The GMOs can be identified specifically by phenotype (aro auxotrophy) and genotype (deletions in *aroC*, *aroD*, *htrA* genes).

b. Techniques used to identify the GMO,
See above.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected).

The purpose of this release is to conduct a phase I clinical trial of the safety and immunogenicity of a single-strain live attenuated typhoid vaccine.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient organism is regularly used, kept or found?

Yes.
Wild-type *S. typhi* are rare in the area of the release. However, it is known that returned travellers from typhoid endemic areas may be rarely colonised by these organisms on their return home and excrete them in their faeces.

3. Information concerning the release and the surrounding area

a. Geographical location (administrative region and where appropriate, grid reference):
The study will involve dosing a number of volunteers with vaccine at Chiltern Clinical Research Unit, Slough, Berkshire (Ordnance Survey grid reference SU 983 793). During the study the volunteers will go about their normal routine and will shed GMOs in their faeces, which will pass into the sewers.

b. Size of the site:

i. The primary release will be made at Chiltern Clinical Research Unit, Slough, Berkshire. The size of the clinical areas in which the GMO will be handled and the volunteers will be dosed is approximately 2500 sq ft.

ii. Wider release area:
Secondary release will occur wherever the recipient volunteers defecate in the days after vaccination. Faeces will be contained and concentrated by the sewer system for treatment and inactivation. It is not possible to estimate the area of this release, however, travel will be relatively restricted due to the requirement for the volunteers to attend the hospital at regular intervals for interview and to provide blood and stool samples.

c. Proximity to internationally recognised biotypes or protected areas (including drinking water reservoirs), which could be affected.
Not applicable - sewage treatment will protect the above.

d. Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO.
Not applicable - see above.

4. Method and amount of release

a. Quantities of GMOs to be released.
Maximum primary release of GMO to all volunteers will be a total of 3.6 x10^{11} cfu. The organisms are expected to replicate for no longer than one cycle and be shed into the sewers over a period of a few days.

b. Duration of the operation.
The release will occur over a period of approximately six months.

c. Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release.
Shedding will be into the sewer system, where normal treatment will contain and inactivate the GMOs.

5. Short description of average environmental conditions (weather, temperature etc..)

Not applicable.

4. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

The actual GMO of this application, ACAM948-CVD, has not been previously administered to human volunteers. However, a strain phenotypically and genotypically identical to the GMO (CVD908-*htrA*) has previously been administered to man in an out-patient clinical trial in the US. There were no serious adverse events recorded in the trial and the strain was not detected in the bloodstream of volunteers. Despite the vaccine strain being shed in the faeces of most recipients for a few days following vaccination, there were no apparent environmental effects nor effects on household members.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Complete name of target organisms.

order and/or higher taxon (for animals)	Mammalia
genus	Homo
species	sapiens
common name	Man

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism.

Transient colonisation of the G.I. tract.

3. Other potentially significant interactions with other organisms in the environment.

None, strains are human specific.

4. Is post-release selection for the GMO likely to occur?

No.

5. Type of ecosystems in which the GMO could be disseminated from the site of release and in which it could become established.

None.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO.

None.

7. Likelihood of genetic exchange in vivo

Two-way exchange of genes may occur with other enteric organisms co-infecting the small intestine of vaccine recipients. This will be a low-frequency event and a full risk assessment suggests no adverse consequences or environmental risks.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in simulated natural environments (e.g. microcosms, etc).

Laboratory experiments in which the replication and survival of the GMOs in chlorinated tap water, river water and sewage have been carried out. Chlorinated water kills 99.9% of the organisms after 4 hours of incubation. The GMOs are killed at a rate of approximately 100-fold every 24 hours in river water and in sewage are killed faster than other bacteria present. The GMOs did not replicate in any of the systems tested.

H. Information relating to monitoring

1. Methods for monitoring the GMOs.

   a. Quantitative culture determinations will be made to estimate the number of GMOs excreted in the stools of some of the vaccine recipients.
   b. The kinetics of inactivation of GMOs in these stool samples by normal tap water and dilute bleach will be determined.
   c. Routine surveillance of sewage treatment and water supplies for bacteria is carried out by the water authorities. If required, the GMOs can be identified by phenotypic or genetic tests as described in D4 above.

2. Methods for monitoring ecosystem effects.

   Not applicable.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

   Not applicable.

4. Spatial extent of the monitoring area (m^2).

   Monitoring as described in 1a and 1b will be conducted in the Clinical Research Unit and associated microbiological laboratories.

5. Duration of the monitoring.

Monitoring as described in 1a and 1b will be carried out for at least 9 days following administration of the last dose of vaccine.

6. Frequency of the monitoring.

Monitoring as described in 1a will be performed daily for 9 days following administration of the first dose of vaccine. Further monitoring will also be performed if volunteers are still excreting vaccine at Day 9.

Monitoring as described in 1b will be performed on selected samples from volunteers over the 9-day monitoring period.

I. Information on post-release and waste treatment

1. Post-release treatment of the site.

None required, release is into sewers and normal treatment will suffice.

2. Post-release treatment of the GMOs.

See 1.

3. a. Type and amount of waste generated.
Human faeces; estimated total amount: 45 kg of waste containing the GMO over a 5-day period following subject vaccination.

b. Treatment of waste.
Normal sewage disposal and treatment.

J. Information on emergency response plans

1. Methods and procedures for controlling dissemination of the GMO(s) in case of unexpected spread.

If a recipient of the GMO develops moderate or severe fever with concurrent shedding or bacteraemia believed to be associated with vaccine administration, the volunteer will be treated as clinically appropriate, including the administration of an appropriate antibiotic.

2. Methods for removal of the GMO(s) from the areas affected.

The GMOs are readily killed by dilute (0.1%) domestic bleach.

3. Methods for disposal or sanitation of plants, animals, soils etc that could be exposed during or after the spread.

Not applicable.

4. Plans for protecting human health and the environment in the event of an undesirable effect.

Any undesirable effects on human health related to inadequate attenuation should be noted in the early phase of the trial, in which cohorts of 5 volunteers will be given sequential, increasing doses of the GMO once the preceding dose level has been

shown to be well-tolerated. Volunteers will be closely monitored clinically and can be treated quickly and effectively should any such untoward effects occur. If any adverse events believed to be related to vaccination occur which cause significant clinical concern regarding the attenuation of the GMO, then the release of the GMO at that particular dose will be halted and the volunteers treated as clinically appropriate, including with an appropriate antibiotic.