Department of the Environment, Transport and the Regions
Advisory Committee on Releases to the Environment
Format 2: Release of genetically modified organisms other than higher plants

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PART B: SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	UK
(b)	Notification number	B/GB/03/35/03
(c)	Date of acknowledgement of notification	16/5/2003
(d)	Title of the project	Live attenuated vaccine for prevention of travellers' diarrhoea
(e)	Proposed period of release	From 01/09/2003 until 30/04/2006

2. Notifier

Name of institution or company: Acambis Research Ltd

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)
RNA virus		(.)
DNA virus		(.)
bacterium		(X)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)

Strain of enterotoxigenic E.coli from which the enterotoxin genes have been removed and chromosomal genes aroC, ompC and ompF have been deleted and protective antigen CS1 (from another ETEC strain) added.

(c) Genetic stability – according to Annex IIIa, II, A(10)
High

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (X)

If yes, insert the country code(s) …

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

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

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

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

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

There is no potential environmental impact of the release of this GMO which is more serious than the release of normal human faecal flora. The GMO is a genetically modified strain of enterotoxigenic *E.coli* (ETEC) designed to be used as a component of a live human vaccine for the prevention of traveller's diarrhoea. The strain has been attenuated by the introduction of large deletion mutations into chromosomal genes and the removal of all known toxin genes. An additional protective antigen, CS1, has been introduced from another E.coli strain.

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 diarrhoea.

The GMO is no more or less able to transfer or receive genes than normal commensal *E.coli*. ETEC strains circulate widely in the world, simultaneously with large numbers of other bacterial enteropathogens (e.g. *S.typhi*, other pathogenic *E. coli, Shigella* sp., *Campylobacter* sp., *Vibrio* sp. etc) but remain a readily recognisable bacterial pathovar. 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, previous clinical trials in human volunteers, using two vaccine strains identically attenuated, revealed no serious adverse events attributable to the vaccine. 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 organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (X)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
  (specify phylum, class) …

other, specify …

2. Name
(i) order and/or higher taxon (for animals) …
(ii) genus Escherichia

(iii)	species	coli
(iv)	subspecies	…
(v)	strain	ACAM2017
(vi)	pathovar (biotype, ecotype, race, etc.)	expresses CFA/II; CS1,2,3
(vii)	common name	E.coli

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	X
Mediteranean	X
Boreal	X
Alpine	X
Continental	X
Macaronesian	X

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (X) No (.)

(d) Is it frequently kept in the country where the notification is made?
Yes (X) No (.)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(X)
soil, free-living	(X)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify …	

(b) If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques

The level of organisms excreted in the faeces of recipient volunteers will be determined by culture on MacConkey agar supplemented with 50μg/ml streptomycin. Coliform

bacterial levels in sewage and water treatment plants are routinely monitored for by water authorities.

(b) Identification techniques

Species can be confirmed as E.coli, if required by commercially available kits such as api20 or api50 (http://www.biomerieux.com/). The GMOs can be identified absolutely specifically by phenotypic and genetic analyses.

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

Yes (X) No (.)

If yes, specify

Non-pathogenic E.coli are designated as category I organisms.

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

Yes (X) No (.) Not known (.)

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

(a) to which of the following organisms:

humans	(X)
animals	(X)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

The GMO is designed to be administered to humans as a component of vaccine to prevent traveller's diarrhoea. As such it is expected to be non-pathogenic for humans as has been demonstrated for two identically attenuated ETEC strains in previous phase I clinical trials. The wild-type strains from which this GMO was derived is only known to colonise humans. The GMO carries no known bacteriophage.

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 (ex vivo).

(b) Generation time in the ecosystem where the release will take place:

Unknown in vivo, approximately 15 mins in exponential growth in rich media.

(c) Way of reproduction: Sexual .. Asexual X

(c) Factors affecting reproduction:

The organisms may colonise and reproduce in the human GI tract. They will be shed in the faeces over a period of up to 4 weeks (predicted median duration 12 days). They are not able to reproduce in simulated environmental conditions and therefore are not expected to multiply following shedding.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	NONE

(b) relevant factors affecting survivability:
The organisms are deficient in the gene aroC 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 country where the notification is made (give notification numbers)
The parent strain, ACAM2007, has been granted permission for release under B/GB/02/R35/01

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i) insertion of genetic material (X)
Plasmid encoding streptomycin resistance as a marker for identification of the GMO. Operon encoding the CS1 antigen from another ETEC strain inserted into the chromosomal ompC locus.

(ii) deletion of genetic material (X)
Plamids encoding enterotoxins and antibiotic resistance markers, chromosomal DNA from aroC, ompC and ompF genes.

(iii) base substitution (.)
(iv) cell fusion (.)
(v) others, specify …

2. Intended outcome of the genetic modification

A live attenuated strain which will form one component of an oral vaccine to protect against diarrhoeal disease caused by wild-type ETEC

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

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (.) No (X)

If no, go straight to question 5.

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

(a) Type of vector

plasmid (.)
bacteriophage (.)
virus (.)
cosmid (.)
transposable element (.)
other, specify …

(b) Identity of the vector

…

(c) Host range of the vector

…

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted

…

(e) Constituent fragments of the vector

…

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify n/a

6. Composition of the insert

(a) Composition of the insert
CS1 operon

(b) Source of each constituent part of the insert
Genomic DNA of another ETEC strain

(c) Intended function of each constituent part of the insert in the GMO
To enable expression of the potentially protective antigen, CS1

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify …

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (X)
If yes, specify …

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

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (X)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)

- other animal (.)
(specify phylum, class) …

other, specify …

2. Complete name

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	Escherichia
(iv)	species	coli
(v)	subspecies	…
(vi)	strain	E1392/75/2A
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	E.coli

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (X) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans (X)
animals (X)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (X) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
The CS1 operon is believed to confer the ability to colonise the small intestine of humans. The modified organism already expresses CS2 and CS3, with similar functions, so that the additional expression is not predicted to materially affect the colonisation ability of the GMO. Other than a possible effect on the ability to colonise humans due to the additional CS antigen expressed, the organism is identical to its parent strain in terms of pathogenicity, toxigenicity, etc.

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (X) No (.)
If yes, specify Non-pathogenic E.coli are classified as category I organisms.

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (X) No (.) Not known (.)

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 recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (X) Unknown (.)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify …

2. Genetic stability of the genetically modified organism
High

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)
The GMO has no pathogenic or harmful characteristics over and above those expressed by its parent strain or other commensal E.coli.

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

in relation to human health -
the toxic or allergenic effects of the non-viable organism and/or its metabolic products,
LPS component of outer membrane toxic if injected

the product hazards,

None known

the comparison of the organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity,
All known pathogenicity determining factors have been removed to create the GMO which is designed as a vaccine, with the exception of the Colonisation Factor Antigens. An additional colonisation factor, CS1, has been co-expressed with the endogenous CS2 and CS3 antigens.

the capacity of the organism for colonisation,
The GMOs are expected to colonise the small intestine of volunteers who are fed them. The additional CS1 expression is considered unlikely to materially affect their ability to colonise human hosts.

if the organism is pathogenic to humans who are immunocompetent -

The GMOs are designed as vaccines 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 methods are used to identify E.coli in the environment.

(b) Techniques used to identify the GMO
The GMOs can be identified specifically by phenotype (Strep. Resistance, aro auxotrophy) and genotype (deletions in *aroC, ompC, ompF* genes)

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
This release is a phase I clinical trial of the safety, ability to colonise recipients and immunogenicity of a component of a multi-strain live attenuated vaccine.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (X) No (.)
If yes, specify: Wild-type ETEC are rare, but not absent in the area of the release. It is known that returned travellers from ETEC endemic areas are often colonised by these organisms on their return home and secrete 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 Barts & The London School of Medicine & Dentistry, in the Clinical Research Centre, 32 Newark Street, London

E1 2AT (Ordnance Survey grid reference TQ346816). 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 (m^2):

(i) actual release site (m^2): The Clinical Research Centre comprises a suite of rooms measuring overall about 60' x 50' (300 m^2)

(ii) wider release site (m^2): Secondary release will occur wherever the recipient volunteers defecate during the course of the study. 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.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
N/A – sewage treatment will protect the above

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
N/A – sewage treatment will protect the above

4. Method and amount of release

(a) Quantities of GMOs to be released:
Maximum primary release to volunteers will be $1x10^{12}$cfu of GMOs. The organisms will replicate to an unknown extent in vivo and be shed into the sewers over a period of a few weeks.

(b) Duration of the operation:
The releases will occur over a maximum period of three years.

(c) Methods and procedures to avoid and/or minimize 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.)
n/a

6. 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.
n/a

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

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Mammalia
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Man

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Colonisation of small intestine

3. Any other potentially significant interactions with other organisms in the environment
None, strain is human specific

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
…

5. Types of ecosystems to 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

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Two way exchange of genes may occur with other enteric organisms co-infecting the small intestine of vaccine recipients.

(b) from other organisms to the GMO:
Two way exchange of genes may occur with other enteric organisms co-infecting the small intestine of vaccine recipients.

(c) likely consequences of gene transfer:

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 stimulated natural environments (e.g. microcosms, etc.):
Laboratory experiments have been carried out in which the replication and survival of the GMO and its wild-type counterparts in chlorinated tap water, river water and sewage slurries has been determined. Chlorinated water kills 90% of the organisms for each 8 hours of incubation. The GMO numbers decrease by at least 1,000-fold after three days in fresh water or sewage slurry.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   a) Quantitative culture determinations will be made to determine the number of GMOs excreted in the stools of recipients of the vaccines
   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 coliform 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
n/a

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
If streptomycin resistant bacteria other than the administered vaccine strain are cultured from the recipients the basis for this resistance will be determined. If it is due to the transfer of plasmid pSTREP from the vaccine strain this can be proven by a combination of plasmid purification, restriction mapping and diagnostic PCR tests. The sequence of pSTREP is available, making such analyses routine.

4. Size of the monitoring area (m^2)
Monitoring as described in 1a and 1b will be done in the Clinical Research Centre and associated microbiological laboratories.

5. Duration of the monitoring
Monitoring as described in 1a will be carried out for 28 days following administration of the last dose of vaccine.

6. Frequency of the monitoring
Monitoring as described in 1a will be performed on days 0, 3, 7, 10, 13, 17, 24, 38 administration of the first dose of vaccine.

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 maximum amount: 200kg over the period of the study.

3. (b) Treatment of waste
Normal sewage disposal and treatment

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
If two or more recipients of the vaccine strain develop significant diarrhoea they will be treated with ciprofloxacin and the further release of that strain will be halted.

2. Methods for removal of the GMO(s) of the areas potentially affected
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
n/a

4. Plans for protecting human health and the environment in the event of an undesirable effect
Any undesirable effects on human health will be noted in the early phase of the trial. Here cohorts of five volunteers will be given sequential, increasing doses of the GMOs once the preceding dose level has been shown to be safe. If there are any adverse events (the worst of which could be diarrhoea) then the release of the particular strain of GMOs will be halted and the volunteers treated appropriately with antibiotics and/or rehydration therapy if required.