COUNCIL DECISION 2002/813/EC

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/15/R47/01/NI
(c) Date of acknowledgement of notification: 9/2/2015
(d) Title of the project: Development of a Combination Vaccine Against Typhoid Fever and Enterotoxigenic Escherichia Coli (ETEC): A Phase 1, Single Centre, Randomised, Double-Blind, Placebo-controlled Clinical Trial to Evaluate the Safety and Immunogenicity of the Oral Live Attenuated Vaccine based on the "Vaxonella" platform technology (Typhetec) at three ascending dose levels
(e) Proposed period of release: From 01/08/2015 until 31/07/2018

2. Notifier

Name of institution or company: Prokarium 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)
Salmonella enterica ssp. *enterica* serovar typhi

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

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.
Negligible

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	Salmonella
(iii)	species	enterica
(iv)	subspecies	enterica
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	Typhi
(vii)	common name	Salmonella Typhi

3. Geographical distribution of the organism

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

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

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

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

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

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	Salmonella Typhi is uniquely adapted to humans

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

…

5. (a) Detection techniques
Microbiological culture

(b) Identification techniques
Selective microbiological media; PCR; agglutination

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify
…

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)

If yes:

(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) of Directive 2001/18/EC
…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Unknown
(b) Generation time in the ecosystem where the release will take place:
Unknown
(c) Way of reproduction: Sexual .. Asexual X..

(c) Factors affecting reproduction:
Organism is an auxotrophic mutant which cannot survive in the environment

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	…	

(b) relevant factors affecting survivability:
…

10. (a) Ways of dissemination
Fecal excretion from vaccinated subjects

(b) Factors affecting dissemination
Normal hygiene procedures make person to person transmission very unlikely

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)
B/10/R40/01

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

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
Expression of additional antigens to make combination vaccine

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 (X) No (.)

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	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)

other, specify …

(b) Identity of the vector

Plasmid pTYPHETEC

(c) Host range of the vector
Gram negative bacteria

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (X) No (.)

antibiotic resistance (.)
other, specify Expression of ETEC specific antigens. No antibiotic resistance genes present

Indication of which antibiotic resistance gene is inserted
None

(d) Constituent fragments of the vector

The pTYPHETEC plasmid possesses a single, 1326 bp synthetic gene encoding the ETEC vaccine protein CF10LTBSTp regulated by the ssaG promoter for expression in the vacuoles of human antigen-presenting cells. The 5' end of the gene encodes the 22 amino acid (aa) cstA signal peptide that directs the CF10LTBSTp protein to the periplasm of ZH9 where it is then cleaved. Following cstA are the first 29 aa of the subunit proteins of 10 ETEC colonisation factors in the following order: CS3-CS5-CS6-CS21-CS2-CS17-CS1-CS14-CS4-CFA/I. These are fused to LT-B (103 aa) which is in turn connected to STp (18 aa) at the 3' end by an 8 aa linker. The other plasmid components are the standard pMB1 medium copy origin of replication and non-expressed par and psi loci for plasmid stability. The pTYPHETEC vector was synthesised chemically.

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

(i) transformation (.)
(ii) electroporation (X)
(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 …

6. Composition of the insert

(a) Composition of the insert

The pTYPHETEC plasmid possesses a single, 1326 bp synthetic gene encoding the ETEC vaccine protein CF10LTBSTp regulated by the ssaG promoter. The 5' end of the gene encodes the 22 amino acid (aa) cstA signal peptide that directs the CF10LTBSTp protein to the periplasm of ZH9 where it is then cleaved. Following cstA are the first 29 aa of the subunit proteins of 10 ETEC colonisation factors in the following order: CS3-CS5-CS6-CS21-CS2-CS17-CS1-CS14-CS4-CFA/I. These are fused to LT-B (103 aa) which is in turn connected to STp (18 aa) at the 3' end by an 8 aa linker.

…

(b) Source of each constituent part of the insert

…

The pTYPHETEC vector was synthesised chemically as fragments.

(c) Intended function of each constituent part of the insert in the GMO

…

The backbone of the plasmid vector is required for replication and expression of the antigen construct. The antigen construct is designed to induce protective immune responses in recipients.

(e) Location of the insert in the host organism

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

(f) 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	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	enterotoxigenic
(ix)	common name	…

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	(.)
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 donated sequences contain non-functional epitopes from 10 colonisation factors from ETEC strains and non-functional components of two toxins, LT and ST.

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 (.) No (X)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (X) 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
Highly stable

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)

(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)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Microbiological culture

(b) Techniques used to identify the GMO
Selective microbiological culture, PCR, agglutination

F. Information relating to the release

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

The release is a Phase 1 clinical trial. If the vaccine is successful it will be an important public health tool.

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 (.) No (X)

If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):

…

BioKinetic Europe Ltd. 14-18 Great Victoria Street, Belfast, BT2 7BA, N.Ireland, UK
The national (OS) grid reference of the proposed site of release is NW 45975 29350.

(b) Size of the site (m^2): n/a m^2

(i) actual release site (m^2): n/a m^2

(ii) wider release site (m^2): n/a m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

…

The site is approximately 1 km from the River Lagan.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

…

N/a; S typhi is uniquely adapted to humans

4. Method and amount of release

(a) Quantities of GMOs to be released:

…

No more than 1×10^{12} CFU.

(b) Duration of the operation:

…

Three years maximum

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

…

Spread beyond the site will only be in the faeces of study subjects. These are expected to be contained and disposed of by the normal sewage system. In the unlikely event of contamination of the environment outside the sewers the GMO will not survive due to its attenuating mutations.

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.

…

No release of this GMO has been made previously. However the parent strain (ZH9) differing only in the absence of the plasmid pTYPHETEC, has been released previously in seven clinical trials in the UK, USA and Vietnam. No health or environmental impacts were observed.

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)	Primate
(ii)	family name for plants	Hominidae
(iii)	genus	Homo
(iv)	species	sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

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

…

Transient colonization of the target organisms (subjects in clinical trial) will induce an immune response designed to confer protection aganst subsequent exposure to pathogenic bacteria.

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

…

N/a

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:
…
Low

(b) from other organisms to the GMO:
…
Negligible

(c) likely consequences of gene transfer:
…
Expression of the ETEC antigens by other gut bacteria, should it occur, would have no negative effects.

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.):
…
Studies of the survival of the parent of the GMO in raw sewage, river water, seawater, soil have been described in the main application. In all cases there was no proliferation and the strain ceased to be detectable after a few days.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
…
N/a

H. Information relating to monitoring

1. Methods for monitoring the GMOs
…
The absence of the GMO in the stools of vaccinated subjects will be confirmed 28 days after the last dose. In the unlikely event that any subjects are still shedding they will be treated with antibiotics until two negative cultures are obtained indicating clearance.

2. Methods for monitoring ecosystem effects
…
N/a

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

…

No routine monitoring for this is planned. However should the need arise the plasmid is readily identified by specific PCR methods.

4. Size of the monitoring area (m^2)

N/a

5. Duration of the monitoring

…

63 Days

6. Frequency of the monitoring

…

Once, unless a positive culture is obtained, in which case it will be repeated as necessary until resolution.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

…

Waste disposal and cleaning will be according to the site's SOPs for handling potentially infectious clinical waste.

2. Post-release treatment of the GMOs

…

GMOs will be captured and neutralized in the sewage system

3. (a) Type and amount of waste generated

…

Disposable clinical containers will be used to prepare the vaccine doses. The amount will be typical for the clinical site operations amounting to a few clinical waste bags per day.

3. (b) Treatment of waste

…

Normal clinical waste disposal as per SOPs.

J. Information on emergency response plans

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

…

The GMO cannot survive or replicate outside of the human host. Stringent precautions are in place to avoid spread form vaccinated subjects to others and such spread has never been noted in previous trials with the parent organism.

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

…

Not required

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

…

Not required

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

…

The health of the volunteers in the trial will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the study physicians as appropriate.