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 — United Kingdom
(b) Notification number — B/GB/19/DR/001/W
(c) Date of acknowledgement of notification — 15/17/2019
(d) Title of the project
A Phase I, randomised, double-blind, placebo-controlled, parallel group dose escalation study to evaluate the safety, tolerability and immunogenicity of three doses of a potential oral enteric fever vaccine (ZH9 + ZH9PA) in healthy subjects 18 to 45 years of age.

(e) Proposed period of release — From 01/09/2019 until 30/09/2021

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 — Phylum: Proteobacteria
Class: Gamma proteobacteria

(b) Identity of the GMO (genus and species)
Genus: *Salmonella*
Species: *enterica*

(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?
ZH9 Yes (X) ZH9PANo (X)
If yes:
- Member State of notification GB
- Notification number B/10/R40/01

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.
As the GMO is specifically adapted to persist in humans, it is considered that the environmental impact as a result of this release is minimal. The natural habitat of the GMO and the parent strain is humans. Humans will be the only recipients of the GMO.
The GMO may be released into the sewage system. Potentially contaminated faeces released into the public mains sewers will be treated according to standard sewage treatment procedures.
It is possible that some of the shed organisms could enter environmental niches other than the sewage system, e.g. soil and water bodies, if a breach of the sewage system were to occur or if faecal samples containing the GMO were disposed of via facilities that do not involve a mains sewage system.
Studies performed to investigate the survivability of the parent strain (ZH9) in soil and aqueous environments showed that S. Typhi carrying the attenuating mutations does not persist, surviving for a limited time only.

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) Phylum: Proteobacteria
Class: Gamma proteobacteria

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	*Salmonella*
(iii)	species	*enterica*
(iv)	subspecies	*enterica*
(v)	strain	Ty2
(vi)	pathovar (biotype, ecotype, race, etc.)	Typhi
(vii)	common name	ZH9

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 Human-restricted

(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:
Not applicable

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

(b) Factors affecting dissemination
Normal hygiene procedures make person to person transmission very unlikely.
Effective sewage treatment processes

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)
GB/ 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	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)

(v) others, specify …

2. Intended outcome of the genetic modification
Attenuation of both ZH9 and ZH9PA. 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 (.) 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 …

6. Composition of the insert

(a) Composition of the insert
The FliC gene of ZH9 (H:d) is replaced by the counterpart gene from S Paratyphi A (H:a)
The LPS locus of ZH9 is modified by the deletion of the rfbE gene and its replacement by a spacer insert comprising the wbdR gene.

(b) Source of each constituent part of the insert
Chemical synthesis

(c) Intended function of each constituent part of the insert in the GMO
Expression of the S Paratyphi A LPS O-side chain and Flagellin determinants

(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 Salmonella
(iv) species enterica
(v) subspecies enterica
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar Paratyphi A
(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 (.) No (X) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
…

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 Class 3

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 GMO will be administered to healthy participants in a Phase I placebo-controlled clinical study. The clinical protocol will be submitted to the Medicines and Healthcare products Regulatory Agency (MHRA) as part of a clinical trials application (CTA). The purpose of the study is to investigate the safety of and immune responses to ascending doses of the GMO, or placebo in healthy participants.

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):
The site of release is **Simbec-Orion Clinical Pharmacology, Simbec House, Merthyr Tydfil Industrial Park, Pentrebach, Merthyr Tydfil, Mid Glamorgan, CF48 4DR, United Kingdom**

The site is located in an urban commercialised area at national (OS) grid reference of SO 064033.

(b) Size of the site (m^2): Not applicable
(i) actual release site (m^2): Not applicable
(ii) wider release site (m^2): Not applicable

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable – the site is an urban commercialized area approximately 200 m from the River Taff.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable. The organism is restricted in its host range to humans.

4. Method and amount of release

(a) Quantities of GMOs to be released:
No more than 4×10^{11} cfu.

(b) Duration of the operation:
The scheduled period of release is from September 2019 until September 2021.

(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.)
The average environmental conditions of South Wales are as follows: maritime climate, characterised by weather that is often cloudy, wet and windy but mild.

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.
The ZH9 strain (the parent of ZH9PA) 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)	Primates
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	Sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Humans

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
It is anticipated that following oral ingestion by the human participants the GMO will reach the small intestine and interact with the intestinal mucosa such that a host immune response is generated against the GMO. It is anticipated that this immune response will be protective against wild-type S Typhi and S. Paratyphi A infection.

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:
Negligible

(b) from other organisms to the GMO:
Negligible

(c) likely consequences of gene transfer:
None

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 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.

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

5. Duration of the monitoring
Until the last subject produces negative cultures

6. Frequency of the monitoring
Once for each subject, 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

- Laboratory waste (plastic ware, liquid reagents, residual GMO).
- Clinical waste (faecal, urine, blood samples; sharps).
- Miscellaneous waste (disposable clothing, tissues).

Small amounts of waste, which can be handled by standard procedures, will be generated.

3. (b) Treatment of waste
All waste disposal and decontamination procedures will be performed using appropriate personal protective equipment, in accordance with local documented procedures for Infection Control. Where applicable, waste (e.g. plastic ware, liquid reagents, microbial cultures, blood, stool and urine samples) will be pre-disinfected by full immersion in 1% Virkon (or 2% Virkon if heavy contamination) for at least 2 hours. All waste, including pre-disinfected material, will be placed in biohazard bags and disposed of as clinical waste according to local standard operating procedures. Used sharps will be discarded straight into a sharps container at the point of use, prior to disposal as clinical waste.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
If any of the participants vomit following administration of the GMO at the clinical site, this will be treated as a biological hazard. Suitable personal protective equipment and disinfectant will be used in the inactivation of the hazard. All resulting waste will be disposed of into sealed containers for autoclaving and incineration, in accordance with local documented procedures for waste disposal and for the management of patients with vomiting and diarrhoea. However, since the GMO is severely attenuated it will not survive outside the human host.

All study samples and specimen sample bags must be labelled with a 'Danger of Infection' label and transported in accordance with local documented procedures.
Wild-type S. Typhi is a human-specific pathogen with no animal, plant or insect vector. The GMO is a severely attenuated form of S. Typhi unable to infect or colonise healthy adults. In an emergency situation measures will be put in place to identify persons who are susceptible to infection with the GMO, may have become infected with, or are carriers of the GMO.
The GMO is sensitive to ciprofloxacin, an antibiotic that is licensed for human use in the event of infection with *Salmonella* sp. This antibiotic is effective in the treatment of acute infection and eliminating chronic carriage. In the case of children, alternative effective antibiotics (e.g. ampicillin, trimethoprim/sulphamethoxazole) are available.
Prophylactic antibiotics can also be used in exposed individuals before infection has been established.

2. Methods for removal of the GMO(s) of the areas potentially affected
Contaminated areas may be decontaminated by the use of standard disinfectants.
Transmission of the organism within the environment is readily controlled by sewage treatment processes.

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
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.