PART 1 (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/10/R40/01
(c) Date of acknowledgement of notification .17./12./2010….
(d) Title of the project Understanding typhoid disease after vaccination: A single centre, randomized, double-blind, placebo-controlled study to evaluate M01ZH09 in a healthy volunteer challenge model with the licensed Ty21a vaccine as a positive control…
(e) Proposed period of release From 01/03/2011 until 01/03/2014.

2. Notifier

Name of institution or company: Emergent Product Development UK 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)
Enterobacteriaceae: *Salmonella enterica* …

(c) Genetic stability – according to Annex IIIa, II, A(10)
PCR evidence indicates that the two large well-characterised attenuating deletion mutations

in the chromosome of the GMO are stable over at least 10 rounds of passaging (approximately 300 generations, well in excess of the number of generations during the manufacturing process, which is approximately 32 generations from Master Cell Bank to end of production). Not only are the genetic modifications of the GMO stable over this period, but the phenotypic characteristics expected of a strain harbouring a deletion mutation in the *aroC* gene also remain the same (ie auxotrophic requirements). The presence of the deletion mutations is confirmed for each batch of the GMO manufactured

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.

The GMO is a severely attenuated strain of a human-specific pathogen (the parent strain) intended for use as an oral vaccine against typhoid fever in healthy adults and children. The GMO will be given orally to volunteers who are likely to shed the organism in stools at low levels for no longer than 17 days. This will constitute the release of the organism resulting in the release into the sewage system. Normal basic hygiene precautions, namely the use of toilets and hand washing, are considered sufficient to prevent person to person transmission.

Wild-type *S*. Typhi (the parent strain) has no animal reservoirs and it does not persist in the environment. The GMO therefore will not persist in the environment as it does not have a selective or survival advantage.

The potential for genetic exchange with any other organisms in the environment is extremely low, given that the GMO does not contain any plasmids (or antibiotic resistance markers), will not persist in the subjects and does not persist in the environment. The GMO is not expected to be present in subjects beyond 17 days post-vaccination and therefore is not expected to be present when the subjects are challenged with wild-type *S.* Typhi (a potential source of complementing wild type genes) 30 days after administration of the GMO. Even if the GMO and the wild type *S.* Typhi strain

were present in the subjects at the same time, the risk of genetic transfer would still be considered very low. The health of the volunteers will be carefully monitored as their safety is paramount in this study.

The GMO has previously been tested in a number of studies in healthy human adult and child volunteers and has been shown to be safe, well tolerated and not persistently shed. It is not likely that the organism will become more persistent or invasive when administered to the healthy adult subjects in the proposed study.

For the purpose of the release, the GMO will be given to a maximum of 60 subjects at a single site in Oxford. The subjects will be healthy adult volunteers. The GMO will be shed in stools for up to 17 days after administration and therefore the subjects will not be permitted to travel outside England for 21 days following each dosing day.

The information presented in this application demonstrates the safety of the GMO, in terms of its inability to cause harm and to persist in the environment. Safety will again be assessed during the proposed clinical trial. The risk assessment shows a low hazard associated with administering the organism to these human volunteers, the risk to other humans is considered to be negligible and the risk to the environment is considered to be effectively zero.

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)	Prokaryote
(ii)	genus	Enterobacteriaceae: *Salmonella*
(iii)	species	Salmonella enterica

(iv)	subspecies	*Salmonella enterica* subspecies *enterica* serovar Typhi
(v)	strain	Ty2
(vi)	pathovar (biotype, ecotype, race, etc.)	Not known
(vii)	common name	*S.* Typhi Ty2

3. Geographical distribution of the organism

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

(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 (.)
(iii) Not known (X)

(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

- 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	humans

- If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques
Culture

(b) Identification techniques

Agglutination for *S. typhi* specific antigens
Biochemical profiling (e.g. API Kit)
Colony blot hybridisation/PCR

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

The recipient/parent strain (*S*. Typhi Ty2) is classified as a human pathogen.
Public Health (Control of Disease) Act 1984
Public Health (Infectious Diseases) Regulations 1988
Genetically modified organisms (Deliberate Release) Regulations 2002
Control of Substances Hazardous to Health Regulations 2002
Categorisation of biological agents according to hazard and categories of containment (Second Supplement to fourth edition 1995) 2000
ACDP Approved List of Biological Agents. Published by HSE, 2004

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:
S. Typhi is human specific; the generation time in vivo is not known; the generation time *in vitro* under laboratory growth conditions is approximately 40 minutes.

(b) Generation time in the ecosystem where the release will take place:
The release will initially be via ingestion of the GMO by subjects in the clinical study. As a result of shedding in faecal stools the GMO could be released into the sewage system following administration to humans. Wild type *S. typhi* is effectively contained and cleared by normal sewage treatment processes. The GMO, a severely attenuated form of *S. typhi*, will not have a survival advantage in this environmental niche

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

(c) Factors affecting reproduction:

The parent/recipient strain (*S.* Typhi Ty2) is a wild type virulent strain of *S.* Typhi which is able to undergo replication *in vivo* without specific nutrient supplements

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	not known

(b) relevant factors affecting survivability:
…

10. (a) Ways of dissemination
Faecal shedding

(b) Factors affecting dissemination
Wild type S. Typhi does not persist in the environment. The survival of the GMO under a range of environmental conditions has been investigated by Emergent Product Development UK Ltd and the data have shown that the GMO does not persist in untreated sewage, in river water, in seawater or in soil

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/../../…

1. A previous phase I clinical study conducted with the GMO itself was given permission by the SGHMS GMSC committee, as follows (this would have gone to HSE ACGM, as part of the cumulative Annual Return):-

SGHMS GMSC GM Number: 99.01

Organism: Group 1

Project: Type A (small scale, research)

No notification number (study conducted prior to introduction of current Deliberate Release regulations)

2. Notification by the same applicant for a related *S.* Typhi vaccine strain (phase I clinical study): **B/GB/02/R37/01**

3. Notification by the same applicant for a related *S.* Typhi immunotherapy strain (phase I clinical study): **B/GB/02/R37/02**

4. Notification by the same applicant for a related *S.* Typhi immunotherapy strain (phase II clinical study): **B/GB/06/R40/01**

5. Notification by another applicant (Acambis Ltd) for a typhoid vaccine candidate strain (ACAM948-CVD) derived from the same parent strain (*S.* Typhi Ty2) as the GMO (clinical study): **B/GB/03/R35/02**

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The intended outcome was rational attenuation of the parent/recipient strain (*S.* Typhi Ty2), via the introduction of two chromosomal deletion mutations, to generate the GMO (*S.* Typhi (Ty2 *aroC*$^{-}$ *ssaV*$^{-}$) ZH9). As a result of the two deletion mutations, the GMO is unable to survive and replicate in human host cells. When delivered to humans, the GMO is intended to generate protective immune responses against *S.* Typhi antigens and thus protect against typhoid fever....

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.

The parent/recipient strain (*S.* Typhi Ty2) is a wild type virulent strain of *S.* Typhi which is able to undergo replication *in vivo* without specific nutrient supplements.

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	(x)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify	Electroporation

6. Composition of the insert

(a) Composition of the insert

The GMO does not contain any functional insert as such. The only inserted sequences present in the GMO are short synthetic DNA sequences, as follows:-

- an insertion of 6 bp at the site of the *aroC* gene deletion
- an insertion of 16 bp at the site of the an *ssaV* deletion

(b) Source of each constituent part of the insert

The short inserted sequences present in the GMO are synthetic in origin

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

- The 6 bp insert at the site of the *aroC* gene deletion is a restriction endonuclease recognition sequence
- The 16 bp insert at the site of the *ssaV* gene deletion consists of two 6 bp restriction endonuclease recognition sequences, separated by a 3 bp stop codon, plus one additional base pair

(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 (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify Not applicable (there is no donor organism)

2. Complete name

(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 …

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

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (.) 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 (.) No (.)

If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) 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 (x) No (.) Not known (.)

Specify: The GMO is severely attenuated compared to the parent/recipient strain (*S.* Typhi Ty2), which decreases survivability in the human host. The GMO will not have a competitive or survival advantage compared to wild-type S. Typhi in the environment.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (x) No (.) Unknown (.)

Specify: The GMO is not expected to differ from the parent strain in terms of the mode of reproduction. The *aroC* deletion present in the GMO results in a requirement for an external source of aromatic compounds for growth, thereby preventing growth or decreasing the rate of growth under certain conditions.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (x) No (.) Not known (.)

Specify: The *ssaV* deletion present in the GMO prevents survival and replication in macrophages and consequently prevents systemic spread *in vivo*

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (x) No (.) Not known (.)

Specify: The GMO is attenuated compared to the parent/recipient strain (*S.* Typhi Ty2) and is therefore unable to infect human cells and cause typhoid fever

2. Genetic stability of the genetically modified organism

PCR evidence indicates that the two large well-characterised attenuating deletion mutations in the chromosome of the GMO are stable over at least 10 rounds of passaging (approximately 300 generations, well in excess of the number of generations during the manufacturing process, which is approximately 32 generations from Master Cell Bank to end of production). Not only are the genetic modifications of the GMO stable over this period, but the phenotypic characteristics expected of a strain harbouring a deletion mutation in the *aroC* gene also remain the same (ie auxotrophic requirements). The presence of the deletion mutations is confirmed for each batch of the GMO manufactured.

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

Culture

(b) Techniques used to identify the GMO

Agglutination for *S.* Typhi specific antigens

Biochemical profiling (e.g. API Kit)

Colony blot hybridisation/PCR

Culture in the presence and absence of a supplement of aromatic compounds

F. Information relating to the release

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

Clinical study to investigate the immune responses, including potential protective effect, to the GMO, to placebo and to a licensed oral typhoid fever vaccine in healthy volunteers.

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: In the proposed study the GMO will be purposefully administered to human volunteers

3. Information concerning the release and the surrounding area

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

The address of the proposed site of release is as follows:-Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LJ.The national (OS) grid reference of the proposed site of release is SP543060.
As a consequence of release the GMO may be released into the sewage system in England; most likely this will be primarily in the Oxfordshire area. The sewage treatment system is designed to contain and clear bacteria, including *Salmonella*.

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): … m^2

The size of the room at the site where the GMO will be administered to study subjects is not known

(ii) wider release site (m^2): … m^2

Oxfordshire

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
The site is approximately 3 km from the River Isis (Thames) and approximately 2 km from the River Cherwell.

(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:

A maximum of 180 volunteers will be included in the study. Of these, a maximum of 60 volunteers will be dosed with the GMO prior to challenge with the wild type *Salmonella* Typhi (Quailes strain). The maximum release of the GMO in the study overall will be no more than 1 x 10^{12} CFU.

(b) Duration of the operation:

The clinical study is expected to begin in March 2011. The first subject is expected to be dosed shortly after the study start date; recruitment may take up to 36 months; the last dose of GMO to be administered to a subject will therefore occur before March 2014. The GMO will be given orally to subjects who are likely to shed the organism in faeces at low levels for no longer than 17 days. This shedding constitutes the release of the GMO, thus the release will end within 17 days of the last dose being administered to the last subject (March 2014). The duration of the release (dosing/shedding phase of the study) will be no longer than 36 months.

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

Methods and procedures at the site of release

All clinical staff directly involved with the study will be strongly encouraged to be vaccinated against *S.* Typhi and will be registered with the University occupational health service prior to commencing work related to the study. Personal protective equipment will be used as appropriate (laboratory coat, apron, safety glasses, and disposable gloves). Only authorised and trained staff will be permitted to enter the clinic rooms and laboratory.

The GMO will be administered in a designated room with separate hand washing facilities. Following dosing with the GMO, volunteers will stay at the Centre for Clinical Vaccinology and Tropical Medicine under observation for 60 mins. The waste from the sanitary facilities at the site enters directly into the public sewers which are capable of containing the organism. After dosing, all surfaces will be disinfected according to documented local procedures. All clinical waste will be inactivated by autoclaving prior to incineration, again according to documented local procedures.

Methods & procedures beyond the site of release

The release of the GMO into the environment will effectively occur after volunteers have been dosed and have left the clinical site, when the GMO is expected to be shed in stools for a period of up to 17 days. The GMO is therefore likely to enter the sewage system during this period. It has been shown that the vaccine strain does not survive in untreated sewage and the sewer is engineered to contain waste prior to processing.

In view of the low infectivity of *S*. Typhi and the level of hygiene and sanitation in the UK, secondary transmission of *S*. Typhi to household contacts or other close contacts is considered highly unlikely. It is thought that *S.* Typhi is virtually never transmitted by direct faecal-oral contact, due in part to the requirement for ingestion of a high inoculum of *Salmonella* Typhi bacteria in order to cause clinical disease.

Volunteers will be educated in hand-washing to avoid secondary transmission of *S.* Typhi and will also receive guidance on procedures for dealing with soiled materials and spillages in a domestic setting so as to minimise secondary transmission.

Food handlers are excluded from the proposed study. Potential participants employed in clinical or social work with direct contact with young children or vulnerable patients or persons in whom typhoid infection would have particularly serious consequences also represent an increased risk and are excluded (unless willing not to work from the point of dosing until demonstrated not to be infected with *S.* Typhi, in accordance with guidance from the Health Protection Agency).

In the proposed study, all participants will be treated with antibiotics, at the latest at 14 days following challenge with the wild type *S.* Typhi Quailes strain; this will lead to the rapid clearance of bacteria.

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

Environmental conditions will be those of the sewage system.

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.

Six clinical studies have previously been conducted with the GMO; in the UK, the United States and Vietnam. In these studies, the GMO was administered to a total of 356 healthy adult and paediatric subjects, as follows: 9 healthy adult subjects in a UK clinical study (study MS01.01); 230 healthy adult subjects in three clinical studies in the United States (studies MS01.03, MS01.04, MS01.13); 16 healthy adult subjects in Vietnam (study MS01.07) and 101 healthy paediatric subjects in Vietnam (study MS01.08). In all studies the GMO was administered in a single oral dose; the maximum dose given in any study was 1.7×10^{10} CFU.

All studies included comprehensive monitoring for the safety and tolerability of the GMO based on clinical, microbiological, haematological and biochemical measurements, and the side effect (adverse event) profile. In particular, subjects were closely monitored for any signs or symptoms of infection with the GMO. All studies also included monitoring for release of the GMO via faecal shedding; the frequency and duration of stool sampling for culture varied from study to study.

The results of the studies overall demonstrate that the GMO was well tolerated, with a good safety profile across the dose range tested (10^7 CFU to 1.7×10^{10} CFU). There were no serious adverse events considered to be related to the GMO. No bacteraemias occurred in any subject. The GMO was adequately immunogenic as assessed by determination of anti- *S.* Typhi responses.

The shedding profile of the GMO is considered to be well-characterised. The results of monitoring for faecal shedding of the GMO across the studies overall show that in the majority of subjects (348/356), the GMO was not shed in stools beyond 7 days after dosing. In total 8 of the 356 subjects were found to shed *S.* Typhi beyond Day 7 post-dosing. Two of these were found to shed to Day 9, 3 to Day 11, 1 to Day 14 and 2 to Day 17. No subjects were found to shed beyond Day 17 and all subjects shedding beyond Day 7 were asymptomatic. When antibiotic treatment was not required per the clinical protocol, faecal shedding was found to resolve in all subjects without intervention.

The results of clinical studies previously conducted with the GMO therefore demonstrate no negative impact of previous releases, either on the environment or on human health.

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)	The target organism is humans
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(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)

Human volunteers ingest the GMO orally. It is anticipated that the organisms will reach the small intestine and will be taken up by specialised antigen presenting cells in which the *S.* Typhi antigens will be expressed. Consequently it is anticipated that the host will mount an immune response against the GMO.

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

Wild-type *S.* Typhi has a very narrow host range and can not colonise any organisms other than humans.

A consideration the GMO is faeco-oral transmission from the volunteers to non-target hosts. Strict exclusion criteria have been set for the trial including criteria to minimise the risk of transmission of the GMO, and in particular to minimise transmission to potentially vulnerable groups. The relevant criteria are:

- Female participant who is pregnant, lactating or who is unwilling to ensure that they or their partner use effective contraception one month prior to vaccination and continue to do so until two negative stool sample obtained a week apart, a minimum of 1 week after completion of antibiotic treatment, has been obtained

- Current occupation involving clinical or social work with direct contact with young children (defined as those attending pre-school groups, nursery or aged less than2 years or highly susceptible patients or persons in whom typhoid infection would have particularly serious consequences (unless willing not to work from point of vaccination until demonstrated to not be infected with *Salmonella* Typhi in accordance with guidance from the Health Protection Agency).
- Current occupation as a commercial food handler (involving preparing or serving unwrapped foods not subjected to further heating).
- Household contact with a young child (defined as those attending pre-school groups, nursery or those aged less than 2 years)
- Household contact who is immunocompromised (e.g., AIDS, chemotherapy*)*

All volunteers enrolled into the trial will also be instructed to maintain strict personal hygiene and proper hand washing will be taught and reinforced, to minimise the risk of faeco-oral transmission. Following administration of the challenge organism, volunteers will be issued with information on enteric precautions (a sheet of detailed instructions for hand washing and other hygiene measures).

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

   The organism will disseminate into the sewage system. The sewage treatment system is designed to contain and clear bacteria, including *Salmonella*. In the unlikely event that the organism reaches other aspects of the environment it will not persist. Wild type *S. typhi* does not persist in the environment and the GMO does not have a survival or selective advantage over wild type *S. typhi*.

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)	information not available
(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:
   Very low since the GMO does not contain any plasmids.

   (b) from other organisms to the GMO:

The potential for genetic exchange in vivo is extremely low given that the propensity of *S*. Typhi for genetic exchange is extremely low, and given that the GMO will not persist in the subjects.

There is very little genetic variation within the global *S.* Typhi population and data indicate that evolution in *S.* Typhi is dominated by genetic drift and loss of gene function rather than by diversifying selection or gain of function through point mutation, recombination or acquisition of new sequences.

The likelihood of the GMO acquiring copies of the deleted *aroC* and *ssaV* genes to regain a virulent phenotype, via genetic exchange with a donor organism, is considered to be negligible. The two deletions in the GMO, both of which independently attenuate the strain, are physically separated on the chromosome by 793 kb. While it is theoretically possible that both mutations could be complemented, this is extremely unlikely, as it would require two separate complementation events. In support of this, *S.* Typhi strains carrying single mutations in *aroC* have been administered to humans in a number of clinical trials and reversion to virulence has never been reported. Similarly, reversion of the GMO to virulence as never been observed following administration to human subjects. Furthermore, normal gut flora does not contain the *ssaV* gene.

In the planned clinical trial subjects will be immunised with the GMO and 28 days later challenged with a wild type *S.* Typhi strain (Quailes strain). The GMO is not expected to be present in subjects beyond 17 days post-vaccination and therefore is not expected to be present when the subjects are challenged with wild-type *S.* Typhi. If the GMO and wild type strain, as a potential source of complementing wild type genes, were present in the subjects at the same time however, then the risk of genetic transfer would still be considered very low. The arguments presented above with regards to the lack of evidence of genetic exchange in *S.* Typhi, the absence of plasmid DNA and the physical separation of the two attenuating deletion mutations on the chromosome within the GMO will apply.

- likely consequences of gene transfer:

The risk of the GMO acquiring copies of the deleted *aroC* and *ssaV* genes to regain a virulent phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible.

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.):
Information not available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not known/not applicable.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The GMO has been constructed so as not to cause harm, and it will not to persist in the environment. The GMO can be detected and identified (monitored) by a combination of methods including *in vitro* culture methods, slide agglutination, biochemical profiling and PCR assay. A colony blot hybridisation technique has been developed that can specifically detect and quantify *S.* Typhi strains in complex samples, such as untreated sewage. The GMO can be uniquely identified by genetic analysis.

Safety monitoring of the volunteers in the proposed clinical study is a vital aspect of monitoring for the GMO. The GMO will be given orally in a single dose to volunteers, who will have regular safety assessments throughout the study. These will include close clinical monitoring for any adverse effect of dosing.

Data on adverse events and systemic symptoms will be collected after vaccination with the GMO and reviewed at follow-up visits. Any subject reporting significant illness felt to be related to infection with the GMO will be investigated, managed and treated as appropriate.

Following challenge with the wild type strain subjects will be monitored for signs and symptoms of clinical infection with *S.* Typhi. If at any time a subject is considered to be clinically infected they will be treated with antibiotics. All subjects will, in any case, receive antibiotic treatment at 14 days following challenge with the wild type strain which will rapidly clear the bacteria. Following 14-days antibiotic treatment, further stool cultures will be performed and, for the infection to be considered cleared, stool samples must be negative for *S.* Typhi on two consecutive occasions (three occasions for health and social care workers).

Clinical results previously obtained for the GMO and two related strains of *S.* Typhi have indicated that shedding of the GMO in faeces may occur for up to 17 days following dosing. Shedding is transient, as the GMO is severely attenuated and unable to infect or colonise the human host. Due to the extensive data on shedding of the GMO collected in previous clinical trials, it is considered that the shedding profile of the GMO is now sufficiently well-characterised and monitoring of stool samples for the presence of the GMO itself is therefore not proposed at any point in this study.

2. Methods for monitoring ecosystem effects

Regular check monitoring of public mains water by water supply companies is in place in England to monitor for potential environmental contamination, and they will respond as per regulations for any coliform bacteria.

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

Not Applicable. The GMO contains no donated genetic material

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

… m^2

Not applicable.

5. Duration of the monitoring

Monitoring will take place for the duration of the clinical study. The study is expected to begin in March 2011, the first subject being dosed shortly after the study start date. Recruitment

may take up to 36 months; the last dose of GMO to be administered to a subject will therefore occur before March 2014. The study will include long term follow-up visits up to 3 years post challenge.

6. Frequency of the monitoring

The proposed trial will include close clinical monitoring of immunised volunteers for any adverse effect of dosing. Volunteers will be vaccinated on study day -28 and challenged with wild type *S.* Typhi on study day 0.

The scheduled follow-up visits during the vaccination phase are on days -21, -18 and -14. The scheduled follow-up visits during the challenge phase are on days 1 to 14. In addition, volunteers will record oral temperature and safety data will be collected via Diary Card between visits.

Volunteers will have 24-hour access to a study physician from time of vaccination until the subject is deemed to be fully treated for S. Typhi. Following challenge with wild type *S.* Typhi, participants will be encouraged to contact one of the study investigators if they develop symptoms of typhoid between the regular reviews. All volunteers will be required to carry a mobile phone which must be switched on at all times, for the first 14 days following challenge.

The study includes long-term follow-up visits at days 21, 28, 60, 90, 180 and at 1, 2 and 3 years post challenge.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Following each release event, the dosing area will be cleaned and disinfected. Any equipment used for dosing will be cleaned and decontaminated or disposed of, as appropriate. All disinfection, decontamination and disposal procedures will be performed wearing suitable personal protective equipment in accordance with documented local procedures including those for Infection Control.

2. Post-release treatment of the GMOs

There will be no post-release treatment of the GMOs. As a consequence of release, the GMOs may disseminate into the sewage system. The sewage treatment system is designed to contain and clear bacteria, including *Salmonella*. In the unlikely event that the GMOs enter the wider environment they will not persist. Regular check monitoring of public mains water by water supply companies is in place in England to monitor for potential environmental contamination, and they will response as per regulations for any coliform bacteria.

3. (a) Type and amount of waste generated

Small amounts of waste of the types listed below, which can be handled by standard procedures, will be generated.

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

3. (b) Treatment of waste

Where applicable, waste will be pre-disinfected by full immersion in appropriate disinfectant for at least 2 hours. All waste, including pre-disinfected material, will be placed in biohazard bags and autoclaved prior to removal from the site and final disposal by incineration.

- Laboratory waste: Laboratory waste will be placed in a container and autoclaved, prior to being incinerated on site.
- Clinical waste: Blood, stool and urine samples collected into lidded, plastic containers for analysis will be autoclaved and then incinerated. Used sharps will be discarded straight into a sharps container at the point of use, prior to disposal.
- Miscellaneous waste: All items that have the potential for contamination, such as disposable clothing, tissues etc will be placed in sealed bags/containers prior to autoclaving and incineration.

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 volunteers 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.

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) are available.

Prophylactic antibiotics can also be used in exposed individuals before infection has been established.

Effective, licensed vaccines against *S.* Typhi are available and could be used to prevent infection if future exposure was thought likely.

Contaminated areas may be decontaminated by the use of appropriate disinfectants.

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

Contaminated areas may be decontaminated by the use of appropriate disinfectants. For example, alcohol wipes, chlorine-releasing tablets or Virkon solution depending on the volume involved and the type of area affected. As a consequence of release the GMO may be released into the sewage system where it will be contained and cleared by normal sewage treatment processes.

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

Treatments with disinfectants, incineration or autoclaving are all effective means for decontamination of exposed items

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

If any of the volunteers vomit following administration of the GMO, this will be treated as a biological hazard. Suitable personal protective equipment and disinfectant will be used to inactivate 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.

The proposed clinical trial procedures involve close clinical monitoring of volunteers for any adverse events, should an illness occur that is felt to be related to infection with the GMO, the subject will be treated with antibiotics as considered appropriate.

In the challenge phase of the study, all volunteers who have not developed typhoid fever following challenge with wild type *S.* typhi Quailes strain, and have therefore not received antibiotics by day 14 post challenge will be treated with oral ciprofloxacin 500mg twice daily for 14 days at that time. Any volunteer in whom a contra-indication to ciprofloxacin develops will be given appropriate alternative licensed oral antibiotic therapy.

The protocol allows for admission of volunteers as inpatients to the John Warin Ward in cases of severe typhoid fever and other circumstances, based on the judgment of the Investigator and study team.

Stool culture for carriage of S. Typhi

In the challenge phase of the study, stool samples for culture will be obtained 3 weeks after completion of the antibiotic course (whenever initiated) and then weekly until 2 successive samples are negative (3 samples for health and social care workers). Once this criterion is satisfied the participant will be considered to be fully treated for *S.* Typhi infection and no longer pose an infection risk. If samples remain positive for *S.* Typhi 4 weeks after completion of antibiotics, then the participant will be referred to a Consultant in Infectious Diseases (Oxford Radcliffe Hospitals NHS Trust) for further management.

In view of the fact that the participants will receive wild type *S.* Typhi, the Proper Officer/Health Protection Unit will be informed of all participants in whom clearance of *S.* Typhi has been demonstrated and of any participant who fails to demonstrate clearance either after the initial 14 day course of antibiotics. In addition, the employer of any participant involved in the provision of health or social care to vulnerable groups will be notified in writing once 3 successive samples are negative.

In addition to the above information, a risk assessment has been carried out in accordance with the Genetically Modified Organisms (Contained Use) Regulations 2000 (as amended) and work with the GMO was classified as a GM class 1 activity (of no or negligible risk).