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/16/R48/01
(c)	Date of acknowledgement of notification	08/June/2016
(d)	Title of the project	

Investigating the role of typhoid toxin in the pathogenesis of enteric fever: A double-blinded, randomised, outpatient human challenge study.

(e) Proposed period of release

From 1st of September 2016 until 1st of March 2018

2. Notifier

Name of institution or company: Oxford Vaccine Group (University of Oxford, UK)

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)

PCR evidence indicates that the three attenuating deletion mutations in the chromosome of the GMO are stable (SB6000 Δ *cdtb*, Δ*pltA* and Δ *pltB*). The phenotypic characteristics expected of a typhoid toxin deficient strain also remain stable as assayed by microbiological assays (growth and purity testing), nucleotide sequencing and typhoid toxin activity assays. The presence of the deletion mutations is confirmed in each batch of GMP manufactured GMO (in addition to microbiological limit and antibiotic sensitivity testing).

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.

This is the first description of the GMO and its use in a clinical study with healthy adult volunteers. The potential environmental impact of the release of the GMO is thought to be negligible as the bacterium will be inactivated by normal sewage and water treatment processes. The wild type (parent strain) *Salmonella* Typhi Quailes has no known animal reservoir and it does not persist in the environment at appreciable levels.

The GMO and the wild type (parent) strain are intended for use as an oral challenge agent in a controlled human infection model in healthy adult volunteers. The GMO and wild type (parent) strain will be given orally to study participants who are likely to shed the organism in stool samples, which will constitute release of the organism into the sewage system. Person to person transmission will be prevented by enhanced hygiene practice until clearance is confirmed (primarily hand washing, the use of toilets and avoidance of food handling). All microbial challenge studies are conducted according to guidelines from the Academy of Medical sciences and subject to approvals from a designated National Research Ethics committee.

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after typhoid diagnosis.

Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative, according to Public Health England guidelines. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Trust) for further management. No instances of stool shedding of the *S*. Typhi Quailes strain after treatment or transmission to secondary contacts were detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of the name, address and date of birth of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants GP will also be notified at the time of stool shedding clearance. In addition, any breaches in enteric precautions that result in another individual coming into contact with the excreta of a participant will be reported to Public Health England.

The potential for genetic exchange with any other organisms in the environment is extremely low as the GMO does not contain any plasmids or antibiotic resistance markers. *S.* Typhi is highly monomorphic, meaning there is very little genetic variation within the global *S.* Typhi population, thereby indicating that its propensity for genetic exchange is extremely low. This is supported by a study that analysed of the whole genomes of 19 *S.* Typhi strains and identified only 1,954 single nucleotide polymorphisms (SNPs) between all of them; approximately 1 every 2,300 bp. Further, very little evidence of recombination between *S.* Typhi isolates or with other bacteria was found. Genomic insertions were rare in the sequenced isolates and evolution in the *S.* Typhi population seems to be characterised by ongoing loss of gene function caused by nonsense SNPs. All data in this study supports the hypothesis 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 risk of the GMO acquiring copies of the deleted *cdtB, pltA* and *pltB* genes to regain a virulent phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible. The GMO does not have a selective or survival advantage in the environment. The GMO is not likely to become more persistent or invasive when administered to healthy adults in the proposed clinical study and pre-clinical studies indicate that Salmonella strains lacking the typhoid toxin have reduced intestinal survival. For the safety of our study participants their health will be monitored very closely (up to daily for the first 14 days post challenge) by our clinical study team.

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) Enterobacteriales
(ii) genus *Salmonella*
(iii) species *S. enterica*
(iv) subspecies *enterica*
(v) strain Quailes
(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 (human restricted pathogen)

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

5. (a) Detection techniques

Microbiological culture

(b) Identification techniques

Selective microbiological culture and biochemical profiling; PCR; agglutination for specific *Salmonella* Typhi antigens

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
Salmonella Typhi is uniquely adapted to humans (human restricted pathogen) and is a causative agent of typhoid fever (also known as enteric fever).

Public Health (Control of Disease) Act 1984
Public Health (Infectious Diseases) Regulations 1988
Genetically Modified Organisms (Deliberate Release) Regulations 2002
Environment Protection Act 1990
Control of Substances Hazardous to Health Regulations 2002
ACDP Approval List of Biological Agents (Published by HSE) 2013

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:

Salmonella Typhi is human specific and the generation time *in vivo* is not known. In the laboratory the generation time under specific growth conditions is approximately 40 minutes.

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

Not known but wild type *Salmonella* Typhi is effectively contained and inactivated by the normal sewage treatment process. Survival time of wild type *S*. Typhi in sewage is usually less than one week. The GMO will not have a selection or survival advantage compared to the wild type (parent) strain.

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

(c) Factors affecting reproduction:

The recipient Quailes strain is a virulent strain of *Salmonella* Typhi which is able to undergo replication *in vivo* without a requirement for 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

(b) Factors affecting dissemination

Dispersal of wild type *Salmonella* Typhi occurs via faeco-oral transmission (contamination of food or water with faeces of infected individuals).

In this release, the GMO will be excreted directly into the sewage system and it is expected that it will be contained there to be subject to normal sewage processing treatments. It is expected, based on evaluation of shedding in previous clinical trials, of the wild type *S*. Typhi Quailes strain, that the *S*. Typhi GMO will be shed by volunteers for approximately 17 days post-dosing.

Strict exclusion criteria have been set for the trial to minimise the risk of transmission of the GMO, and in particular to minimise transmission to potentially vulnerable groups such as immuno-compromised individuals, pregnant women or the very young and elderly. Volunteers will be instructed on how to maintain strict personal hygiene and proper hand washing will be taught and reinforced to minimise the risk of faeco-oral transmission.

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)

None. This is the first description of the *S*. Typhi Quailes GMO strain (SB6000).

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 purpose of the genetic modification is to construct a typhoid toxin-deficient *S*. Typhi Quailes strain. The GMO will be used to investigate the role of typhoid toxin in the pathogenesis of enteric fever in a controlled human infection model. The Oxford Vaccine Group (University of Oxford, UK) has been undertaking controlled human challenge studies using *Salmonella* Typhi and *Salmonella* Paratyphi since 2010. The primary objective of the study is to compare the proportion of participants developing clinical or microbiologically proven typhoid infection following oral challenge with the wild type *S.* Typhi Quailes strain with participants challenged with a typhoid toxin-deficient isogenic mutant of *S.* Typhi Quailes strain SB6000.

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 Bacterial conjugation (to introduce the suicide vector)

6. Composition of the insert

(a) Composition of the insert

The GMO does not contain a functional insert as such. Chromosomal homologous recombination events during the construction of the GMO resulted in *S*. Typhi carrying either wild type copies or a complete deletion of the typhoid toxin genes (*cdtB*, *pltA* and *pltB*). Colonies were screened by PCR and nucleotide sequencing to identity *S*. Typhi mutants carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, generating the SB6000 typhoid toxin deficient *S*. Typhi Quailes strain.

(b) Source of each constituent part of the insert

Step 1: Construction of plasmid pSB5999 containing a complete deletion of the *cdtB* gene:

Two PCR reactions were performed using genomic DNA from *Salmonella* Typhi Quailes as a template using primers designed using the genome sequence of the S. Typhi strain CT18(Accession number AL513382). One PCR amplified immediately 5' of the *cdtB* gene, the other PCR amplified immediately 3' of the *cdtB* gene (1,000 nucleotides respectively). The resulting PCR products were mixed in equimolar concentrations with amplified pSB890 DNA and ligated using the Gibson assembly technique to yield the plasmid pSB5999 (carrying a deletion of the coding sequence of *cdtB*). The Gibson reaction was electroporated into *E. coli* CC118 λ pir with selection on LB agar. Isolated colonies were screened for the presence of the recombinant plasmid. Selected candidate plasmids were sequenced to verify the deletion of *cdtB*. The verified plasmid (pSB5999) was transformed into the *E. coli* strain ß2163 *Δnic35* and selected on LB agar plates to allow the growth of the host bacteria. Plasmid DNA was isolated from the *E. coli* strain ß2163 *Δnic35* and its nucleotide sequence confirmed.

Step 2: Construction of plasmid pSB6000 containing a complete deletion of the pltA and pltB genes

Nucleotide sequences consisting of 2,500 nucleotides immediately 5' and 3' from the coding sequences of the *pltA* and *pltB* genes, respectively, were amplified by PCR from the genome of *Salmonell*a Typhi Quailes strain using primers obtained from the genome sequence of the *S.* Typhi strain CT18 (Accession number AL513382). Two PCR reactions were performed. One PCR product amplified the region upstream of *pltB* whereas the second PCR product amplified the region downstream of *pltA*. The PCR amplification of the suicide vector pSB890 was identical to the PCR described in the construction of the *ΔcdtB* suicide plasmid. The resulting PCR products were purified, mixed in equimolar concentration with amplified plasmid pSB890 and ligated using the Gibson assembly technique to yield the plasmid pSB6000 (carrying a deletion of the coding sequence of both *pltA* and *pltB*). The Gibson reaction was electroporated into *E. coli* CC118 λ Pir and isolated colonies selected on LB agar plates were screened for the desired recombinant plasmid.

A selected candidate plasmid was sequenced to verify the deletion of *pltA* and *pltB*. This plasmid (pSB6000) was transformed into the *E. coli* strain ß2163 *Δnic35* with selecting on LB agar. Plasmid DNA was isolated from the *E. coli* strain ß2163 *Δnic35* and its nucleotide sequence confirmed.

Step 3: Construction of S. Typhi SB5999 strain containing a complete deletion of the cdtB gene

The *E. coli* strain ß2163 *Δnic35* carrying the suicide plasmid pSB5999 was used to introduce the deletion of the *cdtB* gene into the chromosome of the *S*. Typhi Quailes strain by conjugation. A culture of both the donor (*E. coli* strain ß2163 *Δnic35* carrying the suicide plasmid pSB5999) and recipient (*S.* Typhi Quailes) strains were mixed and filtered to facilitate mating. Growth selection for *S.* Typhi carrying plasmid pSB5999 and counter-selection for the integrated plasmid (which excised from the chromosome by homologous recombination) was performed. The recombination event resulted in *S.* Typhi carrying either a wild type copy or a complete deletion of *cdtB* with no plasmid derived sequences left remaining in the bacterial chromosome. Resulting colonies were screened by PCR and nucleotide sequencing to identify *S.* Typhi mutants carrying the expected deletion of the *cdtB* gene (leading to the identification of an *S.* Typhi strain SB5999 carrying a deletion of the entire coding sequence of the *cdtB* gene).

Step 4: Construction of an *S*. Typhi SB6000 containing a complete deletion of the cdtB pltA and pltB genes

The *E. coli* strain ß2163 *Δnic35* carrying the suicide plasmid pSB6000 was used to introduce a complete deletion of the *pltA* and *pltB* genes in the chromosome of the *S.* Typhi Quailes derivative strain SB5999 (carrying a deletion of the *cdtB* gene). Introduction of the plasmid and selection of the transconjugants was performed as described in Step 2. Resulting colonies were screened by PCR. *S.* Typhi mutants carrying the expected deletion of the *pltA* and *pltB* genes yielding the *S.* Typhi strain SB6000 carrying deletions in *cdtB*, *pltA*, and *pltB* genes were identified by nucleotide sequencing.

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

The GMO has been constructed following the deletion of 3 genes from the wild type S. Typhi Quailes strain (deletion of *cdtB*, *pltA* and *pltB* genes). The GMO is a typhoid toxin-deficient *S*. Typhi Quailes strain (SB6000).

The typhoid toxin comprises enzymatic (A) and binding (B) components and is composed of three polypeptide subunits (CdtB, PltA and PltB). The enzymatic component of the toxin is Cytolethal Distending Toxin B (CdtB), a homologue of active A subunit of Cytolethal distending toxin. This toxin is present in several bacterial pathogens and possesses DNAse activity capable of inducing cell-cycle arrest and cellular distension *in vitro*. CdtB is covalently linked to Pertussis-like toxin A (PltA), a homologue of the enzymatic A subunit of pertussis toxin, which has ADP-ribosylase activity. PltA in turn is linked to Pertussis-like toxin B, a homologue of one of the binding (B) sub-units of pertussis toxin. The crystal structure of the typhoid toxin has been resolved and shows the sub-units to be arranged into a relatively unique A_2B_5 structure, comprising two enzymatic A components (CdtB, PltA) and a homopentameric binding B portion (PltB). CdtB is thought to account for the majority of the toxin's enzymatic activity, but all three components are required for toxin delivery and toxicity.

(d) Location of the insert in the host organism

-	on a free plasmid	(.)
-	integrated in the chromosome	(.)
-	other, specify …	(X) Does not apply, no insert. Targeted gene deletion.

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (X, does not apply, no insert)
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)	Enterobacteriales
(ii)	family name for plants	…
(iii)	genus	*Salmonella*
(iv)	species	*S.enterica*
(v)	subspecies	*enterica*
(vi)	strain	Quailes
(vii)	cultivar/breeding line	…
(viii)	pathovar	Typhi
(ix)	common name	*Salmonella* Typhi

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 (.) Not known (X)

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

Salmonella Typhi is uniquely adapted to humans (human restricted pathogen) and is a causative agent of typhoid fever.

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
ACDP Approval List of Biological Agents (Published by HSE) 2013

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: The GMO (Quailes SB6000) will not have a competitive or survival advantage compared to the wild type *S.* Typhi Quailes strain.

(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 (.)

Specific: GMO (Quailes SB6000) is not expected to have a different mode of reproduction from the wild type *S.* Typhi Quailes strain.

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

Specify It is unknown whether the gene deletions in the Quailes SB6000 strain will impact on the survival and consequently systemic spread *in vivo* but it is not anticipated. Evidence from murine studies suggest that the typhoid toxin promotes survival and establishment of persistent asymptomatic infection *in vivo.* As the GMO lacks expression of the typhoid toxin, it is anticipated that survival and dissemination of the GMO in the human host will be impaired.

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

Specify Much of what is known about the pathogenesis of typhoidal *Salmonella* has been inferred from studies using non-typhoidal *Salmonella enterica* serovars that can infect small animals. Administration of purified typhoid toxin in mice recapitulates several of the key features of enteric fever. Pre-clinical studies indicate that the typhoid toxin may play a key role in the pathogenesis of enteric fever and that the unique binding properties of typhoid toxin may, in part, account for the host restriction properties of typhoidal *Salmonella*. Thus, it is anticipated that the GMO lacking expression of the typhoid toxin will have reduced pathogenicity compared to wild type *Salmonella* Typhi.

2. Genetic stability of the genetically modified organism

Genetic stability during the construction of the GMO was observed. Colonies of the GMO were screened by PCR and nucleotide sequencing to identify *S*. Typhi carrying the expected gene deletions.

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

(a) to which of the following organisms?

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

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

Salmonella Typhi is uniquely adapted to humans (human restricted pathogen) and is a causative agent of typhoid fever.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
The wild type and GMO will be detected by microbiological culture.

(b) Techniques used to identify the GMO
The wild type and GMO will be identified by selective microbiological culture; PCR; agglutination for specific *Salmonella* Typhi antigens.

The GMO can be distinguished genetically from the wild type strain by using polymerase chain reaction (PCR) analysis to show the absence of the *cdtB*, *pltA*, and *pltB* genes.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
In this clinical study we propose to utilise the human challenge model established at the Oxford Vaccine Group to further study host-pathogen interactions by investigating the role of the typhoid toxin in the pathogenesis of typhoid fever. This information will be used to inform vaccine design and development potentially influencing public health intervention strategies.

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:
The GMO is human specific and will be administered orally to healthy adult volunteers in a controlled human infection study, according to previously established protocols.

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: Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE. The national (OS) grid reference for the proposed site release is SP543060. As a consequence of shedding through faecal material the GMO may be released into the sewage system in England and primarily within the Oxfordshire area. The sewage system is designed to contain and clear bacteria including *Salmonell*a.

(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 participants is approximately 12.43 m^2

(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 research 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. *Salmonella* Typhi is uniquely adapted to humans (restricted in its host range to humans).

4. Method and amount of release

(a) Quantities of GMOs to be released:

The study will have an enrollment target of 40 adult participants. A target of 20 participants will be administrated the GMO at a dose of $1 – 5 \times 10^4$ colony forming units (CFU). The maximum release of the GMO in the study overall (assuming a 10% withdrawal rate) will be 1.1×10^6 CFU. A substantial proportion of the initial administered dose is unlikely to be shed as viable bacteria in faeces as the GMO and wild type strain will be taken up by host cells in areas of the gut or will die in transit in the gastrointestinal tract. For comparison, 244/274 (89.1%) of stool samples collected during earlier challenge studies were negative for *S.* Typhi following ingestion of the wild type Quailes strain.

(b) Duration of the operation:

The study is anticipated to commence on the 1st of September 2016 with the GMO administered to the first adult volunteer shortly after study recruitment has commenced. The GMO will be given orally and if likely to be shed in faeces at low levels until the completion of a 2 week course of antibiotics. The duration of the release which includes both dosing (challenge period) and shedding phase of the study will be no longer than 18 months (with completion in March 2018).

(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 from stool samples of study participants. These are expected to be contained via disposal by the normal sewage system.

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

Not applicable as the 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.

This is the first description of the GMO and its use in a clinical study with healthy adult volunteers. No release of this GMO has been made previously. However, the wild type (parent) *S*. Typhi strain Quailes has been released previously in four clinical studies conducted within the UK at the Oxford Vaccine Group. All studies include a comprehensive monitoring of the safety of the wild type Quailes strain based on clinical, microbiological, hematology and biochemical measurements. In the proposed study described in this application, clinical study participants will be monitored for signs of infection with the GMO. The results of previous clinical studies using the wild type (parent) Quailes strain demonstrated no negative impact on the environment or on human health (with the exception of causing enteric fever in the controlled human infection model). The potential environmental impact of the release of the GMO is thought to be zero.

The wild type (parent) *Salmonella* Typhi Quailes has no known animal reservoir and it does not persist in the environment in appreciable quantities sufficient to cause disease. The GMO and the wild type strain are intended for use as an oral challenge agent in a controlled human infection model in healthy adult volunteers. The GMO and wild type strain will be given orally to study participants who are likely to shed the organism in stool samples, which will constitute release of the organism into the sewage system. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply further stool samples until proven not to be shedding *S.* Typhi in three consecutive samples. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Trust) for further management. No instances of stool shedding of the *S*. Typhi Quailes strain after treatment or transmission to secondary contacts were detected in previous challenge studies conducted at the Oxford Vaccine Group.

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)

Adult human volunteers will ingest the GMO orally where it will reach the intestine and the host will mount an immune response against the GMO. A proportion of individuals will develop acute typhoid fever following ingestion of the GMO. The attack rate following challenge with wild type S. Typhi at a dose of 10^4 CFU is 65% and it is hypothesized that this will be lower in the individuals challenged with the GMO (attenuated strain). All individuals challenged with the GMO will be treated with a two week course of oral antibiotics (azithromycin 500mg daily), either at the time of acute infection or at Day 14 (whichever is sooner). The GMO has confirmed sensitivity against first, second, third and fourth line antimicrobials. Antibiotic treatment will ensure complete clearance of the GMO, which will be confirmed with collection of serial stool specimens upon completion of treatment.

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

Not applicable as wild type *Salmonella* Typhi has a very narrow host range and cannot colonise any organisms in the environment other than humans. Person to person transmission via the faecal-oral route will be minimised by issuing participants with information on enteric precautions, instructions for participants on obtaining stool specimens, provide the participant with stool sampling equipment. education of participants on correct hand washing techniques (including demonstration and observation), advise participants to inform the study team if any breaches of enteric precautions occur such that another individual comes into contact with excreta from the participant, issue participants with liquid hand soap and paper towels to aid with adherence to enteric precautions.

Strict exclusion criteria have been set for the study including criteria to minimize the risk of transmission of the GMO. Full-time, part-time or voluntary occupations involving: clinical /social work with direct contact with young children (defined as those attending pre-school groups or nursery or aged under 2 years), or clinical/social work with direct contact with highly susceptible patients or persons in whom typhoid infection would have particularly serious consequences (unless willing to avoid work until demonstrated not to be infected with *S*. Typhi in accordance with guidance from Public Health England and willing to allow study staff to inform their employer). If the participant is involved in the provision of health or social care to vulnerable groups then consent will be taken to inform his/her employer of their participation in the study. Full time, part time or voluntary occupations involving: commercial food handling (involving preparing or serving unwrapped foods not subjected to further heating), close household contact with: young children (defined as those attending pre-school groups, nursery or those aged less than 2 years) or individuals who are immunocompromised will all be excluded from taking part in the proposed study.

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. The GMO does not have a selective or survival advantage over wild type *S.* Typhi and will not persist in the environment. The GMO will disseminate into the sewage system which is designed to contain and clear bacteria such as *Salmonella*.

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 known
(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

(b) from other organisms to the GMO:

Negligible

(d) likely consequences of gene transfer:

Negligible risk that the GMO will acquire copies of deleted genes from a donor organism.

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)

Information not known.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Time to onset of stool shedding - time from challenge (Hours/Days) to the first positive stool culture, excluding the first 24 hours following ingestion of challenge agent will be documented. Participants will be required to supply further stool samples until proven not to be shedding *S.* Typhi. Stool samples will be collected at least one week after completion of a 14 day course of antibiotics, until 3 successive stool samples are negative for *S*. Typhi. If persistent stool shedding occurs after completion of antibiotics, participants will be referred to the Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust. Additionally, quantitative stool cultures or PCR may be performed to assess the burden of stool shedding. Isolates from stool samples will be stored frozen for future analysis, which may include phage typing or genetic sequencing.

Blood samples will be monitored daily for the GMO using a combination of microbiological and molecular biology techniques. The Oxford Vaccine Group has developed a fast and highly sensitive novel TSB-bile blood culture-PCR assay which has been used to detect low levels of *S.* Typhi in the blood of participants after challenge. A BACTEC 9240 continuous monitoring system will be used to culture GMO organisms which will be identified as *S*. Typhi via biochemical and serological methods. Confirmed isolates will be tested for antibiotic susceptibility using standard methods.

2. Methods for monitoring ecosystem effects

Not applicable

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

Not applicable (targeted gene deletion)

4. Size of the monitoring area (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 commence on the 1st of September 2016 (pending all necessary approvals) and will run for approximately 18 months (with an expected completion date of March 2018). All study participants will have follow-up visits up to 1 year post challenge.

6. Frequency of the monitoring

Following challenge with the GMO and wild type Quailes strain participants will be monitored daily for the first 14 days post-challenge. Continuous participant safety monitoring will occur throughout the challenge period through a combination of daily clinical review and monitoring of symptoms in an electronic diary.

The protocol for visits will depend on whether the participant develops infection or not. Following diagnosis of enteric fever blood and stool sampling with be performed at 6, 12, 24, 48, 72 and 96 hours post diagnosis. Following completion of antibiotic treatment and confirmed clearance of the GMO in stool samples participants will be monitored a long term follow-up visits at Day 28, 90, 180 and 365. All study participants will agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

An independent Data Safety Monitoring Committee (DSMC) will be established prior to the start of the study. The DSMC will be appointed to provide real-time oversight of safety and trial conduct. The DSMC will have access to data and, if required, will monitor these data and make recommendations to the study investigators on whether there are any ethical or safety reasons why the trial should not continue and will particularly review the wild type *S*. Typhi control group attack rate to confirm the challenge model is proceeding as expected. The DSMC will also be notified if the study team have any concerns regarding the safety of a participant or the general public (e.g. if a participant is not contactable after *S*. Typhi challenge and potentially infectious to others). The outcome of each DSMC review will be communicated directly to the study investigators and documentation of all reviews will be kept in the TMF. The Chair of the DSMC will also be contacted for advice when the Chief Investigator feels independent advice or review is required.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Waste disposal and cleaning will be according to site standard operating procedures (SOPs) for handling both potentially infectious biological and GMO clinical waste. When required the dosing area will be cleaned and disinfected while wearing suitable personal protective equipment in accordance with documented local procedures including those for infection control.

2. Post-release treatment of the GMOs

All clinical and laboratory waste will be treated by according to site standard operating procedures (SOPs) for handling GMO and potentially infectious GMO waste.

Post-release GMO shed in the stool samples of study participants will be eliminated and made safe in the sewage system.

3. (a) Type and amount of waste generated

Disposable laboratory waste containers will be used to prepare the oral challenge preparation. The amount of waste will be typical for the clinical site operations amounting to a few clinical waste bags and bins per day (e.g. faecal, urine, blood samples, disposable clothes, tissues, gloves and aprons). Laboratory waste (e.g. plastic ware, liquid reagents, microbiological waste) will be handled according to local GMO standard operating procedures.

3. (b) Treatment of waste

Normal SOPs will be followed by the site for GMO waste treatment and disposal.

J. Information on emergency response plans

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

Stringent precautions are in place to avoid the spread of the GMO from the study participant to others. Such spread has never been noted in previous studies conducted by the Oxford Vaccine Group using the wild type *S*. Typhi Quailes strain.

Participants who vomit for any reason within 90 minutes of the challenge will be withdrawn from the trial and treated with antibiotics. This will be treated as an emergency spill of the GMO and standard operating procedures will be followed by the research team. Suitable personal protective equipment and disinfectants will be used to inactive the GMO. All waste will be autoclaved prior to disposal according to local GMO standard operating procedures.

Participants will be instructed to notify the study team of any serious adverse events/reactions following administration of the GMO. All participants agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion. A physician from the clinical team will be on-call 24 hours. In addition, participants agree to allow the study team to hold the name and 24-hour contact number of a close friend, relative or housemate who will be kept informed of the study participant's whereabouts for the duration of the challenge period (from the time of challenge until completion of antibiotic course). This person will be contacted if study staff are unable to contact the participant.

Participants will be issued with a Medic Alert-type card containing information including the antibiotic sensitivity of the *S.* Typhi strain (GMO and wild type Quailes strain), study doctor contact details and instruction for the research team to be contacted immediately in the event of illness/accident.

Potential participants with known antibiotic hypersensitivity or allergy to either of the first-line antibiotics (azithromycin, ciprofloxacin or other macrolide antibiotics) will be excluded from the study. The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with side effects. Should an antibiotic cause allergy or intolerance this will be managed by a study doctor and a different antibiotic will be used for subsequent management. The participant's GP will be notified in writing of the antibiotics received. Participants will receive telephone calls or by text messages to remind them to take their antibiotic dose.

There are provisions within the protocol and site facilities to allow for admissions of participants as inpatients to the John Warin Ward (Churchill Hospital, Oxford) in cases of severe typhoid fever and/or other circumstances.

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

All clinical and laboratory waste will be treated by according to site standard operating procedures (SOPs) for handling GMO and potentially infectious GMO waste.

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

All clinical and laboratory waste will be treated according to site standard operating procedures (SOPs) for handling GMO and potentially infectious GMO waste. Such methods of disposal are an effective means to destroy the GMO.

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

The health of our study participants is of the upmost importance and will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the site study physicians as appropriate. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing) (WHO 2003).

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Trust) for further management. No evidence of stool shedding of the wild type *S*. Typhi Quailes strain after treatment or transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants GP will also be notified at the time of stool shedding clearance. In addition any breaches in enteric precautions that result in another individual coming into contact with the excreta of a participant will be reported to Public Health England.

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