Department for Environment, Food and Rural Affairs

Application for consent to release a GMO – organisms other than higher plants

Part A1: Information required under schedule 2 of the Genetically Modified (Deliberate Release) Regulations 2002

Part I

General information

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

Oxford Vaccine Group, University of Oxford
Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
Churchill Hospital
Old Road, Headington
Oxford
OX3 7LE

Chief Investigator: Director of the Oxford Vaccine Group (Oxford, UK).

Principal Investigator: Director of the Oxford Centre for Clinical Tropical Medicine (Oxford, UK).

The Oxford Vaccine Group.

2. The title of the project.

Development of a Live Attenuated Vaccine Against Salmonella Paratyphi A (VASP).

Part II

Information relating to the organisms

Characteristics of the donor, parental and recipient organisms

3. Scientific name and taxonomy.

Name

(i)	order and/or higher taxon (for animals)	Enterobacteriales
(ii)	genus	*Salmonella*
(iii)	species	*enterica*
(iv)	subspecies	*enterica*
(v)	strain	CVD 1902
(vi)	pathovar (biotype, ecotype, race, etc.)	Paratyphi A

(vii) common name *Salmonella paratyphi*

The purpose of the genetic modification is to construct a strain of *S.* Paratyphi A with reduced virulence to investigate its efficacy as a live oral vaccine in a controlled human infection model. The Oxford Vaccine Group (University of Oxford) has been undertaking controlled human challenge studies using *Salmonella* Typhi and *Salmonella* Paratyphi A since 2010. The primary objective of the proposed study is to determine the relative protective effect of two doses of CVD 1902 given 10 days apart compared to placebo (sodium bicarbonate) in a healthy adult *S*. Paratyphi A challenge model.

4. Usual strain, cultivar or other name.

Parent/Recipient strain: *Salmonella* Paratyphi (*S*. Paratyphi) A 9150.

GMO: The GMO is an isogenic mutant of a wild-type *S.* Paratyphi A 9150 containing deletions in the *guaBA* chromosomal operon and the *clpX* gene. The purpose of the genetic modification is to construct a growth deficient attenuated *S*. Paratyphi A strain to act as a live oral vaccine.

5. Phenotypic and genetic markers.

The vaccine strain must be cultured in media supplemented with guanine. Once cultured it can be readily identified as a strain of *S*. Paratyphi A by standard microbiological methods, serum agglutination with antisera specific for O and H antigens and biochemical methods (API, Analytical Profile Index; BioMérieux). Identification of organisms cultured can be achieved by Polymerase Chain Reaction (PCR) to detect the deletions in the *guaBA* chromosomal operon and the *clpX* gene.

6. The degree of relatedness between the donor and recipient or between parental organisms.

The vaccine strain was created by targeted gene deletion therefore there is no donor organism.

7. The description of identification and detection techniques.

The GMO vaccine strain will be detected by microbiological culture using media that is supplemented with guanine. Once cultured, wild type and GMO (S. Paratyphi A) will be identified by biochemical methods and agglutination for specific *S*. Paratyphi A O and H antigens.

The GMO vaccine strain can be distinguished genetically from the wild type *S*. Paratyphi A by using PCR analysis and/or nucleotide sequencing to show the absence of the *guaBA* operon and *clpX* gene.

8. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

The agglutination and biochemical profiling techniques described are routinely used to identify *S*. Paratyphi A strains and together demonstrate 100% specificity.

9. The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey, parasites and competitors, symbionts and hosts.

Enteric fever is a systemic illness caused by infection with human-restricted pathogens *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) and *Salmonella enterica* subspecies *enterica* serovar Paratyphi A (*S.* Paratyphi A).

Enteric fever continues to pose a significant burden of disease with an estimated 14.3 million cases and 135,900 fatalities occurring globally in the year 2017[1]. Up to a quarter of cases may be attributable to *S*. Paratyphi A.

The highest burden of disease is found in low-income countries, where poor sanitation results in the contamination of water and food products and therefore the spread of disease. For reasons not understood the region of highest *S.* Paratyphi A burden appears to be Asia[2]. The demography of those affected by paratyphoid fever is equally not well known, but mortality rates are estimated to be higher in children[1]. Enteric fever caused by either pathogen also remains an important health consideration in developed countries for travellers visiting endemic regions and laboratory workers.

Humans are the only reservoir of paratyphoid infection, with the gallbladder thought to be the main site for long-term carriage. Spread between individuals occurs through faecal-oral transmission, mainly via contaminated food or water. In areas with adequate sanitation (i.e. flushing latrines), person-to-person transmission is rare. Even in the absence of precautions to prevent secondary transmission (as is seen in returning travellers), the rate of transmission of enteric fever causing bacteria is exceptionally low within the UK. In a large recent study of 251 contacts of patients with typhoid fever in London, only one patient was identified as a suspected case of secondary transmission[3]. Similarly, a study in Scotland showed a very low secondary transmission rate in the absence of precautions[4].

10. The organisms with which transfer of genetic material is known to occur under natural conditions.

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

The risk of the vaccine strain acquiring copies of the deleted *guaBA* operon and *clpX* genes to regain a virulent phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible. The vaccine strain does not have a selective or survival advantage in the environment. The vaccine strain is not likely to become more persistent or invasive when administered to healthy adults in the proposed clinical study.

11. Verification of the genetic stability of the organisms and factors affecting that stability.

The guaBA deletion impairs guanine synthesis and strongly reduces the virulence of the closely related *Salmonella* serovar, *S*. Typhi[5]. Deletion of clpX results in the failure of the master flagellum regulator complex flhD/flhC to be degraded resulting in overproduction of flagella and attenuation in vivo in the *Salmonella* serovar S. Typhimurium.

By harbouring two independently attenuating mutations, CVD 1902 provides a high level of safety against the extremely small theoretical possibility of recombinational events occurring that could restore virulence to the vaccine strain.

The presence of the deletion mutations will be confirmed in each batch of GMP manufactured GMO (in addition to microbial limits and antibiotic sensitivity testing).

12. The following pathological, ecological and physiological traits:

a. the classification of hazard according to existing Community rules concerning the protection of human health and the environment

Wild-type *S*. Paratyphi A is an Advisory Committee on Dangerous Pathogens (ACDP) hazard group 3 organism. (ACDP: The Approved List of Biological Agents, Health and Safety Executive (HSE) 2013). At present the GMO vaccine strain CVD 1902 has not received separate ACDP designation.

The Control of Substances Hazardous to Health Regulations (COSHH) 2002 definition of a human pathogen Hazard Group 3 organism: can cause severe human disease and may be a serious hazard to employees; it may spread to the community, but there is usually effective prophylaxis or treatment available.

The GMO vaccine CVD 1902 is significantly attenuated by two independently attenuating mutations and is thus not expected to cause severe human disease. In a Phase 1 study shedding of the vaccine strain was observed up to three days post vaccine dose. Risk of transmission of the vaccine strain will be minimised by participant selection and emphasis on hygiene precautions.

A chronic carrier state, in which *S.* Paratyphi A is excreted in the stools for many years without symptoms, can develop after wild-type *S.* Paratyphi A ingestion. The chronic carrier state is usually seen in older women with pre-existing gallbladder disease (primarily gallstones).

In the absence of effective antibiotic treatment *S*. Paratyphi A can reach the gallbladder and induce an active local infection or exist asymptomatically in a chronic carrier state. Chronic carriage is defined as excretion of *S*. Paratyphi A in stool for more than one year after clinical or sub-clinical infection. The chronic carrier state is thought to be responsible for contributing to the ‘steady-state’ of S. Paratyphi A infection rates seen in endemic settings. Only participants with a normal ultrasound examination of the gallbladder will be included in this study. The likelihood of developing chronic carriage is extremely low, particularly as both the wild type and GMO *S*. Paratyphi A strains are fully susceptible to antibiotics such as co-trimoxazole, ampicillin (amoxicillin) and fluoroquinolones.

b. the generation time in natural ecosystems, and the sexual and asexual reproductive cycle

S. Paratyphi A divides asexually by binary fission and is restricted to a human host. The GMO vaccine strain is a growth-deficient strain of *S.* Paratyphi A which is incapable of sustained intracellular replication due to its specific nutrient requirements (guanine supplementation).

c. information on survivability, including seasonability and the ability to form survival structures, including seeds, spores and sclerotia

Wild type *S.* Paratyphi A is effectively contained and inactivated by the normal sewage treatment process. Survival time of wild type *S*. Paratyphi A in sewage is usually less than one week. *S*. Paratyphi A does not form endospores or any other survival structures. The vaccine strain will not have a selection or survival advantage compared to the wild type strain.

d. pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms

Enteric fever is a systemic illness caused by infection with human-restricted pathogens *Salmonella enterica* subspecies *enterica* serovar Typhi (*S.* Typhi) and *Salmonella enterica* subspecies *enterica* serovar Paratyphi (*S.* Paratyphi).

Humans are the only reservoir of paratyphoid infection, with the gallbladder thought to be the main site for long-term carriage. Spread between individuals occurs through faecal-oral transmission, mainly via contaminated food or water. In areas with adequate sanitation (i.e. flushing latrines), person-to-person transmission is rare.

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Infection in healthy adult volunteers can be achieved by the oral administration of greater than 1000 colony forming units (CFU) of wild-type strain in a bicarbonate solution. Median incubation times and clinical attack rates have been shown to be dose dependent[6].

Following the natural ingestion of *S*. Paratyphi A in contaminated food or water, the bacteria must first pass through the gastric acid environment of the stomach. The bacteria subsequently invade intestinal mucosa via attachment to the M (microfold) cells of Peyer's patches. It is thought that professional phagocytic cells ingest the bacteria within the lamina propria of the intestine. Subsequent dissemination of bacteria contained within macrophages is thought to cause a subclinical primary bacteraemia. This results in dissemination of *S.* Paratyphi A to the liver, spleen, lymph nodes and bone marrow. After a latent incubation period of approximately one week, patients develop symptomatic secondary bacteraemia. Delayed or inadequate treatment can result in the development of complications including gastrointestinal perforation or haemorrhage due to hyperplasia, ulceration and necrosis of Peyer's patches.

The Investigator's Brochure for the GMO vaccine strain CVD 1902 reports two independent *in vitro* invasion assays using a human intestinal epithelial cell line (ATCC # CCL-6), each demonstrating that invasion by CVD 1902 is significantly impaired compared with the ATCC 9150 parent strain. CVD 1902 was shown incapable of sustained intracellular replication, due to its strict requirement for guanine for growth but similar survival in this assay to the licensed attenuated typhoid oral vaccine *S.* Typhi strain Ty21a (an ACDP group 2 organism).

Although not pathogenic when administered orally in mice, IP inoculation of *S*. Paratyphi A can generate a productive lethal infection. This model has been used to compare the relative attenuation of CVD 1902 versus the virulence of the wild type parent strain from which it was derived. The LD_{50} of the wild type *S*. Paratyphi A parent strain ATCC 9150 was <10 CFU, demonstrating a high level of virulence.

In a further mouse study reported in the Investigator's Brochure for the CVD 1902 vaccine the LD_{50} for CVD 1902 was shown to be ~ $10^{6.5}$ CFU, compared with <10 CFU for the wild type parent strain S. Paratyphi A 9150. The LD_{50} for CVD 1902 is approximately one log higher than the LD_{50} of a strain derived from the same 9150 parent carrying the guaBA deletion alone (CVD 1901), and 2 logs higher than the LD_{50} of a strain derived from the same 9150 parent carrying the clpX deletion alone (CVD 1905). This evidence demonstrates that ΔguaBA and ΔclpX are independent attenuating mutations). Introducing into CVD 1902 an expression plasmid carrying both guaBA and clpX fully restored virulence to the level of the wild type parent strain, demonstrating that the two deletion mutations are responsible for the attenuation of CVD 1902.

e. antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy

The vaccine strain does not possess any antibiotic resistance genes. The GMO vaccine strain CVD 1902 is susceptible to ampicillin, ceftriaxone, ciprofloxacin, meropenem and co-trimoxazole thus providing a range of antibiotic agents suitable for human treatment if required.

f. involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration

Not applicable.

13. The sequence, frequency of mobilisation and specificity of indigenous vectors and the presence in those vectors of genes which confer resistance to environmental stresses.

The vaccine strain does not contain indigenous vectors.

14. The history of previous genetic modifications.

The parent wild type *S*. Paratyphi A 9150 strain has not been genetically modified. CVD 1902 was constructed by subsequent genetic deletions of chromosomal gene sequences to first construct CVD 1901 (deleted for the *guaBA* locus) and then CVD 1902 (derived from CVD 1901 by further deletion of the *clpX* locus).These mutations were achieved using lambda red-mediated site-directed mutagenesis. The CVD 1902 GMO vaccine strain has been released previously in a Phase 1 study in the USA. No release of this vaccine strain has been made previously in the UK.

Characteristics of the vector

15. The nature and source of the vector.

The vaccine strain does not contain any vectors. The vaccine strain was generated by the deletion of genetic material. The vaccine strain does not contain a functional insert as such. Chromosomal homologous recombination events during the construction of the vaccine strain resulted in *S*. Paratyphi A carrying either wild type copies or a deletion of the *guaBA* operon and the *clpX gene*).

16. The sequence of transposons, vectors and other non-coding genetic segments used to construct the genetically modified organisms and to make the introduced vector and insert function in those organisms.

The vaccine strain does not contain a functional insert as such. Chromosomal homologous recombination events during the construction of the vaccine strain resulted in *S*. Paratyphi A carrying either wild type copies or a deletion of the *guaBA* operon and the *clpX* gene. Colonies were screened by PCR and nucleotide sequencing to identity *S*. Paratyphi A mutants carrying the deletion of the *guaBA* operon and the *clpX* gene, generating the growth deficient CVD 1902 strain.

17. The frequency of mobilisation, genetic transfer capabilities and/or methods of determination of the inserted vector.

The vaccine strain does not contain any vector derived genetic material. *S.* Paratyphi A mutants carrying the expected deletion of the *guaBA* operon and *clpX* gene yielding the *S.* Paratyphi A strain CVD 1902 carrying deletions in *guaBA* operon and *clpX* gene were identified by PCR.

18. The degree to which the vector is limited to the DNA required to perform the intended function.

The vaccine strain does not contain any vector derived genetic material.

Characteristics of the modified organisms

19. The methods used for the modification.

Standard genetic manipulation methods were used to construct the GMO (including DNA ligation, electroporation, DNA purification, polymerase chain reaction (PCR), bacterial transformation, colony selection and nucleotide sequencing).

20. The methods used:

a. to construct inserts and introduce them into the recipient organism

Parent strain

Salmonella enterica serovar Paratyphi A 9150, lot # 11848, was obtained from the American Type Culture Collection (Manassas, VA).

Construction of CVD 1901 and CVD 1902. Primers needed for the construction of all chromosomal deletions reported here are listed in Figure 1.

Figure 1: List of oligonucleotide primers used in this study.

Name	Sequence[a]	Target
CVOL 13	5'- ATTCCCACTCAATGGTAGC -3'	Ty2
CVOL 15	5'- ATTCCCACTCAATGGTAGC -3'	Ty2
CVOL 26	5'- ATATATATGCGGCCGCTGTAGGCTGGAGCTGCT TC -3'	pKD3
CVOL 27	5'- ATAGGCGCGCCATATGAATATCCTCCTTAGT -3'	pKD3
CVOL 28	5'- CGAACCGTCTGGTTAAGGCGGCTTACGGTAAAAAT TGAGGAAGTTTGAGAGGATAACATGTGAGCGGGAT CAAATTCTAAATCAGCAGGTTATTCAATCGTGTAGG CTGGAGCTGCTTC -3'	pKD3
CVOL 32	5'- TTCATTGATGATGCGGTTGGAAACACGACCCAGGA AGTCATACGGCAGGTGCGCCCAGTGCGCGGTCAT AAAGTCGATGGTTTCGACAGCACGCAGAGAGCAT ATGAATATCCTCCTTAG -3'	pKD3
CVOL 64	5'- CATATGAAGGAGTATTGCCCATGCTACGTATCGCT AAAGAAG -3'	Ty2
CVOL 65	5'- ATGCATCTGCAGTCATTCCCACTCAATGGTAGCC GG -3'	Ty2
CVOL 85	5'- ACAGATAAACGCAAAGATGGCTCGGGCAAA -3'	Ty2
CVOL 86	5'- TTATTCGCCAGAAGCCTGCGCTTCCGGTTT -3'	Ty2
CVOL 87	5'- CCTGAGAATGGCATTTGCGTCGTCGTGTGC -3'	Ty2
CVOL 88	5'- ACGGCGTGTTTACAGGAAAAACGAAAGGGG -3'	Ty2
CVOL 124	5'- GCGGCCGCGAAGGAGTTTGACTCATGACAGATAAA CGCAAAGATG -3'	Ty2
CVOL 125	5'- CATATGTTATTCGCCAGAAGCCTGCGCTTCCGGT TT -3'	Ty2

Deletions were carried out using a modified Lambda Red-mediated site-directed mutagenesis procedure[7]. Briefly, 10 colonies of bacteria carrying Red helper plasmid pKD46 were added to 20ml of 2x soy media supplemented with carbenicillin and L-arabinose (0.2%) and grown at 30°C, 250rpm for 3hrs (OD^{600}nm of ~ 0.6). Bacteria were made electrocompetent by washing 3 times with cold sterile water and concentrating 100 fold. Competent cells were electroporated with 100ng - 1µg of gel-purified PCR product. Following electroporation, bacteria were repaired using 2x soy medium with or without guanine. Cells were incubated in 2x soy media at 37°C for 3 hrs prior to plating on 2x soy agar containing guanine and chloramphenicol (cml) overnight. Antibiotic resistant colonies were selected and screened via PCR for alterations in the chromosomal regions of interest. Positive colonies were restreaked onto 2x soy media containing chloramphenicol, but lacking carbenicillin, to ensure loss of pKD46. Removal of the cml resistance cassette was then carried out using pCP20 encoding FLP recombinase. Colonies exhibiting the desired genotype were re-streaked on 2x soy media lacking antibiotics to ensure the loss of the antibiotic resistance phenotype. Those selected for storage were re-screened via PCR prior to freezing at -70°C in 2x soy media containing 20% (v/v) glycerol.

Step 1: Chromosomal deletion of *guaBA* from *S.* Paratyphi A 9150 to create CVD 1901. The complete genome sequence of *S*. Paratyphi A strain 9150 was not available at the time the gene deletions were made. Since it is critical to have a completely characterized genetic sequence for precise construction of the deletions required for strain constructions the design of all oligonucleotides primers and subsequent DNA templates for chromosomal mutagenesis was based on the annotated *S*. Typhi Ty2 genome sequence (Genbank accession number NC_004631). Subsequent to the construction of CVD 1902, the complete annotated sequence of *S*. Paratyphi A strain 9150 was reported (Genbank accession number NC_006511) and was used to confirm the intended genomic sequence of the completed deletions of *guaBA* and *clpX* in CVD 1902.

The genes which encode inosine-5'-monophosphate dehydrogenase (*guaB*) and guanosine monophosphatesynthetase (*guaA*) form an operon in the chromosome of *S*. Paratyphi 9150. Primers CVOL 28 and CVOL 32 were used for PCR using pKD3 as a template DNA to generate a cassette encoding resistance to chloramphenicol, flanked by ~100bp of chromosomal DNA needed to replace the *guaBA* genes by homologous crossing over. These chromosomal crossovers were catalyzed by the lambda Red recombination system encoded on the temperature-sensitive plasmid pKD46.

S. Paratyphi A 9150 was made electrocompetent and transformed with pKD46 and the *cat* PCR cassette. Transformants were plated at 37ºC, and those exhibiting resistance to chloramphenicol were screened by PCR using CVOL 13 and CVOL 15 which hybridize outside of the *guaBA* region. Unmodified *guaBA* amplified from *S*. Paratyphi A 9150 was found to be ~ 3.5kb, whereas a ~1.4kb fragment was found in two clones carrying the desired replacement of *guaBA* with *cat*. This *cat* gene used to replace *guaBA* was also flanked by a set of direct repeats of a special FLP recombination sequence specifically recognized by an Flp recombinase from *Saccharomyces cerevisiae*.

Since vaccine strains intended for oral vaccination of humans cannot encode resistance to antibiotics, removal of the *cat* resistance cassette was accomplished by introduction of the plasmid pCP20, encoding Flp recombinase, into the *guaBA* mutants by electroporation. Four deletants were analyzed by PCR with primers CVOL 13 and CVOL 15 and found to yield the desired ~ 0.5kb amplicon in comparison with the *guaBA*::*cat* progenitor. One of these *guaBA* deletants of *S*. Paratyphi A 9150 was designated CVD 1901, which was used for all subsequent studies.

Step 2: Chromosomal deletion of *clpX* from CVD 1901 to create CVD 1902. Although extremely unlikely, it is theoretically possible that the engineered auxotrophy in CVD 1901, requiring supplementation with guanine for growth to occur, could be restored by an illegitimate recombination event of unknown mechanism with a *guaBA* genetic sequence of unknown origin. In order to reduce to

an infinitesimally minute probability the chance of reversion of *guaBA*-deleted CVD 1901 to a wild type (wt) genotype, one additional gene, *clpX*, was targeted for deletion as a secondary independent attenuating mutation, thereby creating CVD 1902. *clpX* encodes a chaperone ATPase which works together with a serine protease encoded by *clpP*. Disruption of either ClpX and/or ClpP has been shown to significantly reduce the colonization potential of *Salmonella enterica* serovar Typhimurium in mice. In order to demonstrate the independent attenuating capacity of this deletion, *clpX* was deleted from wild type *S*. Paratyphi A 9150 to create CVD 1905.

To delete *clpX*, primers CVOL 85 and 86 were designed to amplify a ~ 1.3kb fragment encoding *clpX* lacking a start codon from *S.* Typhi Ty2 vaccine strain CVD 908-*htrA*. This fragment was recovered in the plasmid pGEM®-T and subsequently digested with NruI and EcoRI to remove an internal ~ 0.9 kb fragment; the digested termini were treated with T4 DNA polymerase to create blunt ends, and a *cat* cassette encoding resistance to chloramphenicol was inserted. The resulting ~ 1.4kb *clpX*::*cat* cassette was re-amplified using primers CVOL 85 and 86 and electroporated into either *S*. Paratyphi A 9150 or CVD 1901 containing pKD46. As described above with the construction of CVD 1901, transformants were plated at 37ºC, and those exhibiting resistance to chloramphenicol were screened by PCR using primers CVOL 87 and CVOL 88, which hybridize outside of the *clpX* deletion region. Mutants carrying the desired *clpX*::*cat* insertion were then selected for treatment with pCP20 to remove the *cat* cassette. Mutants constructed from either wild type *S*. Paratyphi A or CVD 1901 contained an altered *clpX* gene that produced a smaller ~ 0.5kb amplicon from PCR reactions using primers CVOL 87 and CVOL 88 (Panel A, lanes 1 – 6) compared to ~1.4kb amplicons observed for unaltered *S*. Paratyphi A 9150 (lane 7). PCR analysis of the same strains with CVOL 13 and 15 confirmed that *guaBA* was absent only from *clpX*-deleted strains derived from CVD 1901 to produce CVD 1902 and not from *clpX*-deleted strains derived from *S*. Paratyphi A 9150 which produced CVD 1905.

b. to delete a sequence

See Section 20a.

21. The description of any insert and/or vector construction.

Not applicable, no insert (targeted gene deletion).

22. The purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function.

The genotypic identities of CVD 1901 and CVD 1902 were determined using genomic DNA from CVD 1902 as template in PCR reactions and comparing to wild type *S*. Paratyphi A ATCC 9150 template DNA. Stored frozen vials of CVD 1901 and

CVD 1902 were given to independent investigators for reconfirmation of the genotypic identity by performing PCR to demonstrate the presence and intended sizes of the Δ*guaBA* and Δ*clpX* deletion mutations. Chromosomal sequencing was also performed for both deletions using a stored vial of CVD 1902. For Δ*guaBA*, 1317 bases of sequence upstream of the chromosomal scar region and 1155 bases of downstream sequence were compared to the genome of *S.* Paratyphi A ATCC 9150 (GenBank # CP000026); no mismatches were identified outside of the mutated *guaBA*. For Δ*clpX*, 1142 bases of sequence upstream of the chromosomal scar region and 1130 bases of downstream sequence were compared to the genome of *S.* Paratyphi A 9150; again, no mismatches were identified outside of the mutated *clpX* gene.

23. The methods and criteria used for selection

As described in Section 20a.

24. The sequence, functional identity and location of the altered, inserted or deleted nucleic acid segments in question and, in particular, any known harmful sequence.

No harmful sequences have been introduced into the GMO. The GMO has been constructed following the deletion of 3 genes from the wild type *S.* Paratyphi A ATCC 9150 strain (deletion of *guaBA* operon and *clpX* gene). The GMO is a growth deficient *S*. Paratyphi 9150 strain.

Characteristics of the genetically modified organisms in their final form

25. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed.

The GMO has been constructed following the deletion of 3 genes from the wild type *S.* Paratyphi A ATCC 9150 strain (deletion of *guaBA* operon and *clpX* gene). The GMO is a growth deficient *S*. Paratyphi 9150 strain.

A candidate *S*. Paratyphi A live oral vaccine strain has been engineered by deleting the *guaBA* chromosomal operon (which encodes two enzymes employed in the distal *de novo* guanine nucleotide biosynthesis pathway) and also by introducing a deletion in *clpX* (which encodes a chaperone ATPase that functions with the serine protease encoded by *clpP* to form a complex that participates in a variety of metabolic processes, including playing a role in controlling the availability of regulatory proteins and the breakdown of misfolded proteins). One of the phenotypic consequences of a deletion mutation in *clpX* is the hyperexpression of flagella. Introducing a deletion in *clpX* is intended to provide a second, independent attenuating mutation to minimize the risk of recombinational events that could theoretically restore the wild type genotype. *Salmonella* deleted in clpP, *clpX* (encoding the ATPase), or *clpPX* have diminished ability to produce systemic

infection; yet the resultant *clpPX* mutants remain capable of protecting mice against wild type challenge.

26. The structure and amount of any vector or donor nucleic acid remaining in the final construction of the modified organisms.

There is no vector or donor nucleic acid remaining in the final GMO.

The phenotypic characteristics expected of a growth deficient strain remain stable as assayed by microbiological assays (growth and purity testing) and PCR confirmation of the deletion mutations. The presence of the deletion mutations will be confirmed in the batch of GMP manufactured GMO (in addition to microbial limits and antibiotic sensitivity testing).

28. The rate and level of expression of the new genetic material in the organisms and the method and sensitivity of measurement of that rate and level.

Not applicable (gene deletion).

29. The activity of the gene product.

Not applicable (gene deletion).

30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector.

Both the wild type and GMO strains can be cultured in the laboratory using standard selective media. To support the growth of CVD 1902, standard media must be supplemented with guanine. GMO colonies have the morphological characteristics of wild type *S*. Paratyphi A and can be identified by PCR, biochemical profiling and specific anti-sera against bacterial antigens O and H. PCR and nucleotide sequencing can specifically identify GMO *S*. Paratyphi A mutants carrying the deletion of the *guaBA* operon, and *clpX* genes.

The phenotypic characteristics expected of a growth deficient strain were confirmed by microbiological assays (growth and purity testing). The presence of the deletion mutations will be confirmed in the batch of GMP manufactured GMO in addition to microbial limits and antibiotic sensitivity testing.

31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

The microbiological, biochemical and genetic analyses described in Section 30 enable the GMO to be identified. PCR and nucleotide sequencing can specifically identify *S*. Paratyphi A mutants carrying the deletion of the *guaBA operon*, and *clpX* gene (the growth deficient *S*. Paratyphi CVD 1902 strain).

32. The history of previous releases or uses of the organisms.

The vaccine strain CVD 1902 *Salmonella enterica* serovar Paratyphi A live oral vaccine was administered to humans in a single phase 1 trial conducted at the University of Maryland in the United States (NCT01129453 ClinicalTrials.gov)**.** This trial has not been formally published however the immunogenicity data from the trial were published in 2019[8]. The single-site, randomized, double-blinded, phase 1 study in healthy young adults (18-45 years) was designed to investigate the safety, clinical tolerability, and immunogenicity in a dose-escalating fashion of oral doses of CVD 1902. In this trial 30 recipients received the vaccine in single doses of between 1.56 x 10^6 CFU and 2.25 x 10^{10} CFU. At all dosing levels, CVD 1902 was well tolerated and there were no SAEs attributable to the vaccine and no halting rules were met. Adverse events were experienced by 11 of the 30 vaccine recipients during the 11 days post-vaccination (37%): 21 were graded as mild and 2 were of moderate severity. In comparison, 5 of the 10 placebo recipients experienced a solicited reaction (50%): 7 were graded as mild and 3 were graded as moderate. No fevers were observed that were considered to be related to the vaccine.

Microbiological Responses. No subject had a positive blood culture. Thirteen vaccinees (43%) excreted vaccine after inoculation. The three subjects who met the study definition of diarrhea all shed the vaccine strain. No subject shed vaccine after day 3. No placebo recipients excreted the vaccine, indicating that no horizontal transmission of the vaccine strain occurred from vaccinees to placebo controls, despite their co-habitation within an isolation ward (shared meals and bathroom facilities).

33. In relation to human health, animal health and plant health:

S. Paratyphi A is a human restricted pathogen. There are no anticipated effects on animal or plant health.

a. the toxic or allergenic effects of the non-viable organisms and/or their metabolic products

The vaccine has previously been administered to human subjects in a phase 1 clinical trial conducted at the University of Maryland Center for Vaccine Development in the USA (NCT01129453 ClinicalTrials.gov)**.**

To date no toxic or allergenic effects were reported following administration of the vaccine to adult human volunteers.

b. the comparison of the organisms to the donor, recipient or (where appropriate) parental organism regarding pathogenicity

The wild type parent strain, *S.* Paratyphi A 9150 from which the GMO was derived, is virulent in humans causing the typical symptoms of paratyphoid fever. The GMO is significantly attenuated through deletions in both the *guaBA* operon and the *clpX*

gene. The GMO has been assessed for safety and immunogenicity in a phase 1 clinical study involving 30 healthy adults and was shown to be well tolerated and safe in all subjects. The findings of these studies therefore show that the GMO is has not been shown to cause disease in healthy adult human subjects.

c. the capacity of the organisms for colonization

The GMO is significantly attenuated and did not cause invasive disease at a range of doses as evidenced by the lack of bacteraemia in the Phase 1 study. Shedding was not seen beyond day 3 in clinical study subjects

d. if the organisms are pathogenic to humans who are immunocompetent:

The GMO has not been shown to cause disease in healthy adults in a Phase 1 trial. The *guaBA* operon and *clpX* gene mutations incorporated into *S.* Paratyphi A to generate the GMO are specifically designed to attenuate the ability of the GMO to cause disease and no evidence of invasiveness (i.e. bacteraemia, chronic carriage and/or long-term faecal shedding) was observed in any of the 30 healthy subjects that have been administered the GMO to date. Moreover, the GMO is not expected to be pathogenic in immunocompromised individuals. Pre-clinical experiments performed at the University of Maryland have shown that compared to wild-type *S.* Paratyphi A 9150 the CVD 1902 GMO vaccine strain was incapable of sustained intracellular replication in a human intestinal epithelial cell line (ATCC # CCL-6) due to its strict requirement for guanine for growth. This indicates that the attenuation of the GMO is not dependent on the ability of the innate or specific immune system to control the replication or spread of the organism and it should therefore be attenuated in immuno-compromised individuals.

i. diseases caused and mechanisms of pathogenicity including invasiveness and virulence

The GMO has not been shown to cause disease in healthy adults as evidenced by failure to cause bacteraemia in a Phase 1 study and experimental data in human intestinal epithelial cell lines which show that CVD 1902 is incapable of sustained intracellular replication due to its strict requirement for guanine for growth.

ii. communicability

Despite shedding of the vaccine strain for up to 3 days in those who received vaccine there were no positive stool cultures in the placebo group. Both vaccinees and placebo participants were living on a research isolation ward, sharing bathroom facilities and eating meals together. This suggests that CVD 1902 is not readily transmissible between individuals.

Within this study we aim to characterise if GMO shedding occurs throughout the vaccination period by stool culture. Participants will be given information about hand hygiene and enteric precautions.

iii. infective dose

The GMO has not been shown to cause disease in healthy adults in a Phase 1 trial. The doses employed in the Phase 1 study were 1-5 $x10^6$ to 1-5 $x10^{10}$ CFU in five dose levels. Immunogenicity data showed a greater response at the highest two dose levels and therefore a dose of not less than 1 $x10^{10}$ CFU has been chosen as the dose for this study.

Live oral vaccines such as Ty21a (licensed in the UK) and Vaxchora (CVD103 HgR, licensed in the US) do not have specific doses in terms of CFU. Instead, a dose minimum or range is given (not less than 2 x 10^9 and 4 x 10^8 to 2 x 10^9 CFU respectively). A dose range minimum approach will be taken with CVD 1902. The lower limit of the dose range chosen for this trial (1 x 10^{10} CFU) corresponds with the highest dose category in the Phase 1 trial performed at the Center for Vaccine Development, University of Maryland School of Medicine.

Manufacturing data from three batches of CVD 1902 show viability counts of less than 2.5 x 10^{11} CFU per ml of the finished product. Dose vials contain 2.1ml hence the maximum number of organisms given to each participant in a single dose will be 5.25 x 10^{11}CFU.

Participants will receive two doses of vaccine (or placebo) according to their allocation following randomisation. Participants and study staff conducting visits will be blinded as to their allocation.

For the safety of our study participants their health will be monitored closely, with 6 visits during the vaccination period (including first vaccination, intervals between visits 3-11 days) with blood and stool cultures at each visit. Following challenge with a wild type organism (32 days following last vaccine dose) they will be monitored daily for 14 days by the study team. Blood and stool cultures will be taken at each visit. For a week following each vaccination and during the challenge period (from challenge to day 21 post challenge) participants will be asked to measure their temperature twice daily and record solicited symptoms on an electronic diary, unsolicited symptoms can be recorded at any time up to 28 days after challenge.

iv. host range and possibility of alteration

The GMO is restricted to a human host.

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.* Paratyphi A is highly monomorphic, meaning there is very little genetic variation within the global *S.* Paratyphi A population, thereby indicating that its propensity for genetic exchange is extremely low.

The risk of the GMO acquiring copies of the deleted *clpX* and *guaBA* genes to regain a pathogenic *Salmonella* Paratyphi A 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. For the safety of our study participants their health will be monitored closely, with 6 visits during the vaccination period (including first vaccination, intervals between visits 3-11 days) with blood and stool cultures at each visit. Following challenge with a wild type organism (32 days following last vaccine dose) they will be monitored daily for 14 days by the study team. Blood and stool cultures will be taken at each visit. For a week following each vaccination and during the challenge period (until day 21 post challenge) participants will be asked to measure their temperature twice daily and record solicited symptoms on an electronic diary, unsolicited symptoms can be recorded at any time up to 28 days after challenge.

v. possibility of survival outside of human host

The GMO does not have a selective or survival advantage in the environment. The wild type (parent strain) *S.* Paratyphi A strain has no known animal reservoir and it does not persist in the environment in appreciable quantities sufficient to cause disease. Person to person transmission throughout the trial will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

vi. presence of vectors or means of dissemination

The GMO does not contain a functional vector or insert.

Humans are the only reservoir of wild-type paratyphoid infection, with the gallbladder thought to be the main site for long-term carriage. Spread between individuals occurs through faecal-oral transmission, mainly via contaminated food or water. In areas with adequate sanitation (i.e. flushing toilets), person-to-person transmission is rare. The GMO is intended for use as a vaccine in a controlled human infection model with wild-type *S.* Paratyphi A in healthy adult volunteers. The GMO 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. The GMO will disseminate into the sewage system which is designed to contain and clear bacteria such as *Salmonella*.

Person to person transmission throughout the trial will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing).

vii. biological stability

The phenotypic characteristics expected of a growth deficient strain remain stable as assayed by microbiological assays (growth and purity testing) and PCR confirmation of the deletion mutations. The presence of the deletion mutations will be confirmed in the batch of GMP manufactured GMO (in addition to microbial limits and antibiotic sensitivity testing).

viii. antibiotic-resistance patterns

The vaccine strain does not possess any antibiotic resistance genes. The GMO vaccine strain CVD 1902 is susceptible to ampicillin, ceftriaxone, ciprofloxacin, meropenem and co-trimoxazole thus providing a range of antibiotic agents suitable for human treatment if required.

Participants will not routinely receive antibiotics following vaccination doses. In Phase 1 antibiotics were given to ensure eradication of the vaccine strain before discharge from the research isolation ward. Blood cultures taken at intervals during the vaccination period will be monitored in conjunction with electronic diary temperature recordings for the seven days after each vaccination. Stool cultures will be monitored for shedding but antibiotic treatment will not be given unless there are safety concerns from microbiological data or clinical assessment of the participants.

If a participant became unexpectedly unwell or culture results indicated that antibiotic treatment were necessary following vaccination then treatment would be commenced. This would require the blinding for this particular participant to be broken. Potential participants with known antibiotic hypersensitivity or allergy to either ciprofloxacin, azithromycin or other macrolide antibiotic, co-trimoxazole or ceftriaxone will be excluded. Second line antibiotics such a co-trimoxazole, azithromycin and amoxicillin are available if intolerance or allergy became apparent.

All participants will receive a 7 day course of antibiotics (ciprofloxacin 500mg bd po) following challenge with wild-type *S*. Paratyphi A, at the time of diagnosis with paratyphoid fever or at day 14 (whichever is the first to occur).

To ensure clearance of infection and to exclude carriage, stool samples for culture will be obtained upon completion of the antibiotic course. If clearance samples were found to be positive (defined as stool cultures being positive for *S*. Paratyphi A in at least 1 in 3 stool samples collected at least one week after completion of antibiotics) then an additional antibiotic course will be given. If a participant continues to have positive clearance samples then they will be referred to an Infectious Diseases Consultant the Oxford University Hospitals NHS Foundation Trust for further management.

All positive isolates received during the vaccination period will be assumed to be CVD 1902.

ix. allergenicity and x. availability of appropriate therapies

Hypersensitivity reactions are very unlikely with CVD 1902 due to the nature of the vaccine components (sterile water, bicarbonate and *Salmonella* bacteria) and oral route of delivery.

The antimicrobial susceptibility data of CVD 1902 (section 12e) demonstrates a number of therapeutic options should treatment be required following vaccination. Potential participants with known antibiotic hypersensitivity or allergy to either ciprofloxacin, azithromycin or other macrolide antibiotic, co-trimoxazole or ceftriaxone will be excluded.

If a participant became unexpectedly unwell or culture results indicated that antibiotic treatment were necessary following vaccination then treatment would be commenced. This would require the blinding for this particular participant to be broken. Second line antibiotics are available if intolerance or allergy became apparent.

All participants will receive a 7 day course of antibiotics (ciprofloxacin 500mg bd po) following challenge with wild-type *S*. Paratyphi A, at the time of diagnosis with paratyphoid fever or at day 14 (whichever is the first to occur). 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.

To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk.

Public Health England will be informed of all participants in whom clearance has been demonstrated and of any participant who fails to demonstrate clearance after the initial 7 day course of antibiotics or after any other antibiotic treatment.

The possible adverse effects of CVD 1902, *S.* Paratyphi A infection or the effect of some antibiotics on the outcome of pregnancy are unknown. Therefore, pregnant women will be excluded by history and laboratory tests, and female participants will be specifically excluded if they are not willing to use effective contraception during the vaccination and challenge period of the study until clearance of paratyphoid infection is confirmed. Should pregnancy occur, information about outcome of the pregnancy will be sought.

e. Other product hazards

The GMO has undergone significant attenuation so severe disease in individuals is not anticipated.

The Phase 1 study remains unpublished however immunogenicity data from the trial was published by in 2019[8]. Thirty healthy adult volunteers aged 18 - 45 years received a single dose of CVD 1902 (6 per dose group, dose range 1.7×10^6 – 2.3×10^{10} CFU). Foreseeable reactions to the vaccine include: fever, nausea, vomiting, diarrhoea, anorexia, malaise, abdominal cramps, and headache. Faecal shedding of the CVD 1902 vaccine strain may also occur following vaccination.

In the Phase 1 study the CVD 1902 vaccine was reported to be well-tolerated. There were:

- no SAEs attributable to vaccine
- no fevers observed
- loose stools meeting the definition of diarrhoea in 10% of vaccine recipients
- 5/30 complained of abdominal cramps versus 0/10 placebo controls (statistically non-significant, p=0.31).
- fecal shedding in almost half of vaccine recipients (13/30 shed on at least one day, for a maximum of three days after immunisation)

No participants had a positive blood culture after immunisation and there was no transmission between participants who received CVD 1902 and those who received the placebo control despite being closely housed in a research isolation ward (including sharing meals and bathroom facilities).

In the Phase 1 trial, on day 12 all CVD 1902 recipients started a 14 day course of oral antibiotics. Participants were monitored until day 180 post-immunisation.

The general risks to participants in this proposed study are associated with study-fatigue, phlebotomy, symptomatic infection following challenge with wild-type *Salmonella* Paratyphi A and the small risk of subsequent complications.

Complications of paratyphoid fever, such as perforation or haemorrhage, occur almost exclusively in patients who do not receive appropriate antibiotic treatment for an extended period of time.

During the vaccination period participants will be monitored at clinic visits and remotely via an e-diary. Participants will not routinely receive antibiotics following vaccinations. Following challenge with wild-type *S*. Paratyphi A participants will be closely monitored for 21 days post challenge, until a 7-day course of antibiotic is completed, minimising the risk of complications. Participants in this study will be

treated after developing a fever that persists for 12 hours or if *S*. Paratyphi A is recovered from a blood culture drawn 72 hours post-challenge.

Humans are the only reservoir of typhoid infection, with the gallbladder thought to be the main site for long-term carriage. A chronic carrier state, in which *S.* Paratyphi A is excreted in the stools for many years without symptoms, can develop after *S.* Paratyphi A ingestion. The chronic carrier state is usually seen in older women with pre-existing gallbladder disease (primarily gallstones). Only participants with a normal ultrasound examination of the gallbladder will be included in this study.

Part III

Information relating to the conditions of release

The release

34. The description of the proposed deliberate release, including the initial purpose or purposes of the release and any intention to use the genetically modified organisms as or in a product in the future.

Purpose of the deliberate release: In this clinical study we propose to utilise the *S.* Paratyphi A human challenge model established at the Oxford Vaccine Group (UK)[9] to further study host-pathogen interactions by investigating the efficacy of the GMO CVD 1902 as a live attenuated vaccine to prevent paratyphoid infection. This information will be used to inform vaccine design and development. It has the potential to influence public health intervention strategies. The title of the project is: 'Development of a Live Attenuated Vaccine Against *Salmonella* Paratyphi A'.

The overall aim of this project is to assess the protective efficacy of CVD 1902, a live attenuated oral paratyphoid vaccine using a controlled human paratyphoid infection model. Secondary objectives include the safety and tolerability of the vaccine strain and immune response to the vaccine.

35. The intended dates of the release and time planning of the experiment including frequency and duration of releases.

The study is expected to commence on the 1st of September 2020 (pending all necessary approvals) and will run for approximately 48 months (with an expected completion date of September 2028). All study participants will have follow-up visits up to 1 year post challenge. The study will have an enrolment target of between 66 and 76 adult participants. A target of 33-38 participants will be administered the GMO at a dose of not less than 1 x 10^{10} colony forming units (CFU). Participants in the vaccine arm will receive two doses 10 days apart.

The study is anticipated to commence on the 1st of September 2020 with the vaccine administered to the first adult volunteer shortly after study recruitment has commenced. The vaccine will be given orally and is likely to be shed in faeces after administration by some participants.

The duration of the release which includes both dosing (vaccination) and shedding phase of the study will be no longer than 48 months (with completion in September 2028). Monitoring will take place for the duration of the clinical study. Due to the evolving global situation with respect to coronavirus the duration time for this study is lengthy as it is unclear exactly at what point it will be feasible to commence this study. We anticipate the trial will run for at least 48 months but this may be longer, for example if the start is significantly delayed, if there are difficulties with recruitment or if follow on studies involving the release of this GMO are required, hence release may occur until September 2028.

The data on shedding of the GMO collected in the phase 1 clinical trial show that vaccine strain shedding can be expected for three days, but given the small number of participants in the phase 1 it is possible that shedding may occur for a longer period than this. One of the aims of this study is to further characterise the shedding profile of the GMO. It is therefore proposed to monitor stool samples taken from volunteers in the study for the presence of the GMO at each visit during the vaccination period (baseline (Va), day -35 (Vb), day -32 (Vc), day -25 (Vd), day -18 (Ve), day -7 and at day 0 (challenge day)). However, stool samples will also be monitored for the presence of *S.* Paratyphi A daily for 14 days following challenge with the wild type NVGH308 strain. All isolates cultured from participants' blood or stool during the vaccination period will be assumed to be the vaccine strain CVD 1902.

To minimise accidental transmission of the GMO to surfaces or to other individuals (non-target hosts), the volunteers will be instructed to maintain strict personal hygiene during the study and proper hand washing techniques will be taught and reinforced. Faeco-oral transmission of the GMO from the volunteers to other people is a consideration for this study and strict exclusion criteria have been set to minimise such transmission, in particular to minimise the risk of transmission to potentially vulnerable groups such as small children and those who are immunocompromised.

36. The preparation of the site before the release.

All necessary study approvals must be place before the study can commence. The clinical study site at which the GMO will be administered to participants will be initiated according to Good Clinical Practice (GCP) and according to documented legal and local procedures and guidelines prior to study initiation. All site staff will be given study specific training. There is no licensed vaccine against *Salmonella*

Paratyphi A and typhoid vaccination does not provide cross-protection against *Salmonella* Paratyphi A.

37. The size of the site.

The proposed release will be conducted at the Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE at map reference SP543060. The size of the room at the site where the GMO will be administered to study participants is approximately 12.43m^2 with wider release into the Oxfordshire area (via faecal shedding into the sewage system).

38. The method or methods to be used for the release.

The GMO vaccine strain will be administered orally in a sodium bicarbonate solution (to neutralise gastric acid) allowing the bacterial to survive initial entry into the gastrointestinal tract. The outpatient model for the release of GMO enteric fever pathogens has been demonstrated to be safe and acceptable to participants[9]. The GMO will disseminate into the sewage system which is designed to contain and clear bacteria such as *Salmonella*.

39. The quantity of organisms to be released.

The study will have an enrollment target of 66-76 adult participants. A target of 33-38 participants (those in the vaccine arm) will be given two doses of the GMO of not less than 1 x 10^{10} colony forming units (CFU) per dose. A substantial proportion of the initial administered dose is unlikely to be shed as viable bacteria in faeces as the vaccine strain will be taken up by host cells in areas of the gut or will die in transit in the gastrointestinal tract. Shedding of the GMO was not detected beyond 3 days post-vaccination in the original phase 1 study.

40. The disturbance of the site, including the type and method of cultivation, mining, irrigation, or other activities.

Not applicable.

41. The worker protection measures taken during the release.

All clinical and laboratory staff will be appropriately trained according to GCP, GCLP, GMO risk assessments and local standard operating procedures (SOPs), including infection control, transportation of the GMO/associated clinical samples and GMO waste disposal policies. All study staff handling the GMO and who have direct contact with the study participants will use personal protective equipment (aprons and gloves) as appropriate. Existing SOPs govern the safe and accurate conduct of all study procedures in addition to a study-specific Clinical Study Plan.

All staff members working at the release site will be informed of the commencement of the challenge study and use of the GMO. GMO exposure records for all staff members will be recorded in association with the University of Oxford Safety Office.

42. The post-release treatment of the site.

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

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

43. The techniques foreseen for elimination or inactivation of the organisms at the end of the experiment or other purposes of the release.

Local and site SOPs will be followed for inactivation of the GMO waste prior to disposal. Post-release GMO shed in the stool samples of study participants will be eliminated and made safe in the sewage system.

It is possible that some of the shed organisms could enter environmental niches other than the sewage system, e.g. soil and water bodies, if a breach of the sewage system were to occur or if faecal samples containing the GMO were disposed of via facilities that do not involve a mains sewage system. Studies performed to investigate the survivability of the GMO in soil and aqueous environments show that the GMO does not persist, surviving for a limited time only.

Regular monitoring of water coliform count by Public Water Supply Companies is in place to monitor for potential environmental contamination, and they will respond as per standard operating procedures for any coliform bacteria.

44. Information on, and the results of, previous releases of the organisms and in particular, releases on a different scale or into different ecosystems.

The GMO has previously been administered to 30 human subjects in a phase 1 clinical trial conducted at the University of Maryland Center for Vaccine Development in the USA (See Item 32).

The environment (both on the site and in the wider environment)

45. The geographical location and national grid reference of the site or sites onto which the release will be made, or the foreseen areas of use of the product.

The proposed release will be conducted at the Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE at map reference SP546059.

46. The physical or biological proximity of the site to humans and other significant biota.

The site is in central Oxford, in an urban residential area known as Headington.

47. The proximity to significant biotopes, protected areas or drinking water supplies.

The research site is approximately 3 km from the River Isis (Thames) and approximately 2 km from the River Cherwell.

48. The climatic characteristics of the region or regions likely to be affected.

Not applicable.

49. The geographical, geological and pedological characteristics.

Not applicable.

50. The flora and fauna, including crops, livestock and migratory species.

Not applicable. The GMO (and wild type strain) are restricted to a human host.

51. The description of target and non-target ecosystems likely to be affected.

As a consequence of the release, the GMO will be released into the public sewage treatment system. 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 process.

The wild type (parent strain) *S.* Paratyphi 9150 has no known animal reservoir and it does not persist in the environment at appreciable levels.

52. The comparison of the natural habitat of the recipient organisms with the proposed site or sites of release.

The natural habitat of *S*. Paratyphi A is humans. Study participants enrolled to the proposed clinical study will be the only recipients of the GMO. As a consequence of the release, the GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes.

All clinical and laboratory waste generated at the release site will be inactivated prior to disposal (according to local GMO standard operating procedures, legal and University policies). All site staff will be trained in infection control procedures.

53. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

None known.

Part IV

Information relating to the interactions between the organisms and the environment

Characteristics affecting survival, multiplication and dissemination

54. The biological features which affect survival, multiplication and dispersal.

S. Paratyphi A is host-restricted and only infects humans. The GMO does not have a selective or survival advantage in the environment compared to the wild type strain. 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 growth deficient *Salmonella* strains have reduced intestinal survival.

The risk of the GMO acquiring copies of the deleted *guaBA* operon and *clpX* gene genes to regain a normal growth phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible.

In the absence of effective antibiotic treatment *S*. Paratyphi A can reach the gallbladder and induce an active local infection or exist asymptomatically in a chronic carrier state. Chronic carriage is defined as excretion of *S*. Paratyphi A in stool for more than one year after clinical or sub-clinical infection. The chronic carrier state is thought to be responsible for contributing to the ‘steady-state’ of S. Paratyphi A infection rates seen in endemic settings. Only participants with a normal ultrasound examination of the gallbladder will be included in this study. The likelihood of developing chronic carriage is extremely low, particularly as both the wild type and GMO *S*. Paratyphi A strains are fully susceptible to antibiotics such as ciprofloxacin, ampicillin and co-trimoxazole.

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. Survival time of wild type *S*. Paratyphi A in sewage is usually less than one week. It is expected, based on evaluation of shedding in the previous phase 1 clinical trial, using the GMO vaccine strain, that the GMO will be shed by volunteers for at least 3 days post-dosing.

Environmental Survival.

The ability of three strains of *S*. Paratyphi A: wild type ATCC 9150, CVD 1901, and CVD 1902, to survive was determined by microcosm studies performed by the University of Maryland Center for Vaccine Development.

Aqueous microcosms.

Distilled water was taken from sterilized distilled water in the University of Maryland Microbiology laboratory. Tap water was collected from the Baltimore city (CVD laboratory). Stream water was taken from Baltimore County. Raw Wastewater was taken from Back River Wastewater Treatment Plant in Baltimore, USA.

Methods:

1. Strains were grown over night on LB (Lennox) agar plates (Guanine supplemented when needed). Several colonies were resuspended in saline and plated on new Lennox plates and incubated for 24h at 37ºC. Growth was then resuspended in sterile PBS.

2. Turbidity was adjusted to about $3x10^8$ cells per ml.

3. 30ml samples of each water microcosm were inoculated separately with each strain in separate flasks to produce final concentrations of 10^5 cfu/ml (Distilled Water, Tap Water and Stream Water) or 10^6cfu/ml (Raw Wastewater).

4. As controls, un-inoculated water samples were also studied to determine if there was any background growth capable of growth on MacConkey agar.

5. All microcosms were incubated statically for 14 days at room temperature.

6. Samples withdrawn on days 0, 2, 4, 7, 10, 14 and plated for viable count on MacConkey agar (supplemented with guanine if needed).

7. Suspicious colonies were distinguished from all other lactose negative colonies by colony morphology, biochemical reactions with TSI or API, and by agglutination with specific S. Paratyphi A antisera.

Results and Conclusions. All Salmonella strains died off rapidly in Distilled Water, Tap Water, and Stream Water, persisting no longer than 2 days before becoming undetectable (<10 cfu/ml is the limit of detection using 0.1ml samples). These experiments indicate that all *S*. Paratyphi A strains studied would be unlikely to persist in non-sewage environmental water samples. As expected, Raw Wastewater (sewage) had a background growth of lactose negative bacteria. These lactose negative colonies were distinguished from the test strains by colony morphology, biochemical reactions with TSI or API, and by agglutination with specific *S*. Paratyphi A antisera. In this microcosm, both wild type ATCC 9150 and the CVD 1901 vaccine strain were detected on day 10, although viable counts had dropped by 2 logs; no S.

Paratyphi A was recovered by day 14. In contrast, the CVD 1902 candidate vaccine strain was undetectable by day 2. This result suggests that although Raw Wastewater supports the growth of *S*. Paratyphi A to a limited degree, it is not expected to allow sustained growth of the CVD 1902 vaccine.

Soil and Food (Coconut Milk) Microcosms.

Both rich and poor soils were collected from the Baltimore area. 'Trader Joe's' brand Coconut Milk (Product of Thailand Ingredients: coconut milk and water, No preservatives, No artificial colours or flavours) was also studied.
The rationale for testing the growth of the wild type and *S*. Paratyphi A and the attenuated CVD 1901 and CVD 1902 strains in this food is that a large outbreak (167 cases) of paratyphoid A fever occurred in Singapore in 1996, with cases being widely distributed in that city-state and largely confined to one ethnic group[10] in which de-shelled coconut was incriminated as the food vehicle. In that outbreak, an epidemiologic case-control study showed that consumption of food items that contained de-shelled coconut as an ingredient was significantly associated with paratyphoid fever ($p < 0.001$)[10]. When the import of deshelled coconut was thereupon banned, cases were no longer reported by four weeks after the ban.

Methods

1. Strains were grown overnight on LB (Lennox) agar plates (Guanine supplemented when needed). Several colonies were resuspended in saline and plated on new Lennox plates and incubated for 24h at 37ºC. Growth was then resuspended in sterile PBS.
2. Turbidity was adjusted to about $3x10^8$ cells per ml.
3. 30ml samples of Coconut Milk were inoculated separately with each strain in separate flasks to produce final concentrations of 10^6 cfu/ml
4. Sediment-slurries of soils were prepared by mixing 30 grams of soil into 30ml of sterile distilled water. These suspensions were then inoculated separately with each strain to produce final concentrations of 10^7 cfu/ml.
5. As controls, un-inoculated soils and Coconut Milk were also studied to determine if there was any background growth capable of growth on MacConkey agar.
6. All microcosms were incubated statically for 21 days at room temperature.
7. Samples were withdrawn on days 0, 3, 4, 7, 10, 14, 21, and plated for viable counts on MacConkey agar (guanine supplemented when needed).
8. Suspicious colonies were distinguished from all other lactose negative colonies by colony morphology, biochemical reactions with TSI or API, and by agglutination with specific *S*. Paratyphi A antisera.

*Results and Conclusions***.** In soils, there was a high background of lactose negative bacteria on the uninoculated control plates. All *S*. Paratyphi A strains were

detectable on day 7, with all strains undetectable on day 10 for poor soil but persisting on day 10 in rich soil; no viable counts were detected by day 21 in either of these microcosms. All the *S.* Paratyphi A strains grew well in the coconut milk. However, the attenuated vaccine strains did not grow more avidly or persistently than the wild type ATCC 9150 strain. It is not known why coconut milk would support the growth of the attenuated strains CVD 1901 and 1902, in which exogenous guanine is required for growth. In summary, in none of these experiments using soil or a food vehicle does either vaccine strain grow better or for a longer period than the wild type *S*. Paratyphi A strain.

55. The known or predicted environmental conditions which may affect survival, multiplication and dissemination, including wind, water, soil, temperature and pH.

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.

56. The sensitivity to specific agents.

The vaccine strain does not possess any antibiotic resistance genes. The GMO vaccine strain CVD 1902 is susceptible to ampicillin, ceftriaxone, ciprofloxacin, meropenem and co-trimoxazole thus providing a range of antibiotic agents suitable for human treatment if required.

Interactions with the environment

57. The predicted habitat of the organism.

The natural habitat of *S*. Paratyphi A is humans. Study participants enrolled to the proposed clinical study will be primary recipient of the GMO. As a consequence of the release, the GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes.

58. The studies of the behaviour and characteristics of the organisms and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses.

Not applicable.

59. The capability of post-release transfer of genetic material:

a. from the genetically modified organisms into organisms in affected ecosystems

The risk of genetic transfer with other organism is extremely low since the GMO does not contain functional insert or plasmid.

b. from indigenous organisms to the genetically modified organisms

The risk of the GMO acquiring copies of the deleted *guaBA* operon *and clpX* gene to regain a normal growth phenotype, via genetic exchange (conjugation, transduction) with a donor organism, is considered to be negligible.

60. The likelihood of post-release selection leading to the expression of unexpected or undesirable traits in the genetically modified organisms.

The GMO does not have a selective or survival advantage in the environment.

61. The measures employed to ensure and to verify genetic stability, the description of genetic traits which may prevent or minimise dispersal of genetic material and methods to verify genetic stability.

The guaBA deletion impairs guanine synthesis and strongly reduces the virulence of the closely related *Salmonella* serovar, S. Typhi[5]. Deletion of clpX results in the failure of the master flagellum regulator complex flhD/flhC to be degraded resulting in overproduction of flagella and attenuation in vivo in the *Salmonella* serovar S. Typhimurium.

By harbouring two independently attenuating mutations, CVD 1902 provides a high level of safety against the extremely small theoretical possibility of recombinational events occurring that could restore virulence to the vaccine strain.

The presence of the deletion mutations will be confirmed in each batch of GMP manufactured GMO (in addition to microbial limits and antibiotic sensitivity testing).

62. The routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact and burrowing.

S. Paratyphi A 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, providing the participant with stool sampling equipment, education of participants on correct hand washing techniques (including demonstration and observation), advising 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, issuing participants with liquid hand soap and paper towels to aid with adherence to enteric precautions.

As a consequence of the release in faecal samples, the GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes.

63. The description of ecosystems to which the organisms could be disseminated.

The GMO will be released into the sewage system via faecal shedding from study participants who have received oral doses of the vaccine. The GMO will be released into the public sewage treatment system where it will be inactivated by normal sewage and water treatment processes. Should a breach in the sewage system occur the GMO could enter other ecosystems such as soil and water bodies.

64. The potential for excessive population increase of the organisms in the environment.

Negligible, the GMO will be inactivated by normal sewage and water treatment processes.

65. The competitive advantage of the organisms in relation to the unmodified recipient or parental organism or organisms.

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

66. The identification and description of the target organisms if applicable.

Healthy adult volunteers aged 18-55 years will be the target organism in the proposed clinical study. Strict inclusion and exclusion criteria apply to the study protocol (as described in Section 77).

67. The anticipated mechanism and result of interaction between the released organisms and the target organisms if applicable.

In the proposed study, a controlled human paratyphoid infection model will be used to investigate the efficacy of a live attenuated oral vaccine to protect against paratyphoid fever. It is anticipated that following oral ingestion by the human volunteers, the GMO will reach the small intestine and interact with the intestinal mucosa such that a host immune response is generated against the GMO. It is anticipated that this immune response will be protective against wild-type *S.* Paratyphi A infection to which all participants will be challenged with during the study. Following challenge all participants will be treated with antibiotics, either at the time of diagnosis or at day 14 post wild type challenge (whichever is sooner). The insights obtained from this study will provide data on the validity of this approach and its potential for control of paratyphoid infection. If efficacy is shown in the model, the further development of the vaccine and potential licensure could be based on the data generated in this project.

68. The identification and description of non-target organisms which may be adversely affected by the release of the genetically modified organisms, and the anticipated mechanisms of any identified adverse reaction.

None expected as *S*. Paratyphi A is a human restricted pathogen.

69. The likelihood of post-release shifts in biological interactions or in the host range.

The risk of the GMO acquiring copies of the deleted *guaBA* operon and *clpX* genes to regain a wild-type 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. For the safety of our study participants their health will be monitored closely by our clinical study team by electronic diary reporting, visits and blood and stool sampling.

70. The known or predicted interactions with non-target organisms in the environment, including competitors, prey, hosts, symbionts, predators, parasites and pathogens.

None expected.

71. The known or predicted involvement in biogeochemical processes.

None expected.

72. Any other potentially significant interactions with the environment.

None expected.

Part V

Information on monitoring, control, waste treatment and emergency response plans

Monitoring techniques

73. Methods for tracing the organisms and for monitoring their effects.

The shedding profile of the vaccine strain has been assessed in an initial phase 1 clinical study, and it was established that shedding of the GMO in faeces occurred for no longer than 3 days after administration (see **Item 32**).

In this study adult human volunteers will ingest two doses of the GMO orally where it will reach the intestine and the host will mount an immune response against the GMO. Blood and stool cultures will be taken at day -35 (Vb), day -32 (Vc, second vaccination), day -25 (Vd), day -18 (Ve), day -7 (pre-challenge), Day 0 (challenge), and daily throughout the 14 day post-challenge period and at visits after paratyphoid diagnosis. On challenge day the volunteers will receive 1-5 x 10^3 CFU of the wild-type *S*. Paratyphi A (strain NVGH308).

To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

Blood samples will be monitored for presence of *S*. Paratyphi A using standard microbiological techniques with guanine supplementation (including automated blood culture monitoring). The GMO is growth restricted and therefore requires media to be supplemented with guanine. CVD 1902 and wild-type organisms will be identified as *S*. Paratyphi A via biochemical and serological methods. Confirmed isolates will be tested for antibiotic susceptibility using standard methods.

Extensive assay development work has been performed at the University of Maryland to ensure that the GMO can be isolated by *in vitro* culture methods. Standard media requires supplementation with guanine to support the growth of CVD 1902. Once cultured, positive identification of *S.* Paratyphi A can be confirmed with slide agglutinations and biochemical profiling. A PCR assay can then be applied as required, to distinguish the GMO from wild-type *S.* Paratyphi A.

Regular 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 (The Water Supply (Water Quality) Regulations 2000; SI 2000 No 3184).

74. Specificity (to identify the organisms and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques.

The *S*. Paratyphi A GMO will be monitored as described in Section 73 (standard microbiological techniques for the detection of S. Paratyphi A strains with additional guanine supplementation to media) followed by PCR to distinguish from the wild-type strain. However should this need arise the gene deletions can also be readily identified by nucleotide sequencing.

75. Techniques for detecting transfer of the donated genetic material to other organisms.

Not applicable (targeted gene deletion).

76. Duration and frequency of the monitoring.

Summary

During the proposed clinical trial, volunteers randomised to receive the GMO, CVD 1902 (or placebo) will receive two doses of the GMO (or placebo) on study day -42 and study day -32. They will then receive a single dose of the wild type *S*. Paratyphi A challenge (NVGH308 strain) on study day 0. The proposed trial will include close clinical and physiological monitoring and, following challenge, haematological and biochemical monitoring of volunteers for any adverse effect of dosing.

After dosing with the GMO it is possible that the GMO will be transiently shed in faeces. Clinical data previously obtained for the GMO indicate that shedding may occur for 3 days following dosing (refer to **Items 32** for details). Shedding is transient as the GMO is severely attenuated and unable to multiply within, or colonise the human host. Stool samples will be monitored for the presence of *S.* Paratyphi on D-35, D-32 (second vaccination day), D-25, D-18, D-7 and before challenge (D0) and daily for 14 days following challenge with the wild type NVGH308 strain. Stool samples are also monitored following the completion of antibiotics (three samples taken 48 hours apart, commencing one week after the completion of antibiotics).

Standard microbiological methods will be used to identify *S*. Paratyphi A strains (see section 73) with additional guanine supplementation to the media to support GMO growth. Positive isolates from the vaccination period (ie before challenge with the wild-type strain) will be assumed to be the vaccine strain.

Throughout the study subjects will be monitored in person and remotely for signs/symptoms of ill-health, both for evaluating the safety and tolerability of the initial vaccination (with the CVD 1902, or placebo) and for monitoring subjects for signs and symptoms of a clinical infection with *S.* Paratyphi A following wild-type challenge. After vaccination and challenge, subjects will visit the clinic for regular assessment. They will also be instructed to contact the study team if they become unwell between scheduled visits and be supplied with an emergency telephone number for a study physician providing out-of-hours contact. In this way the subjects are closely monitored and appropriately assessed and treated should they develop signs or symptoms of clinical or bacteriological evidence of, *S.* Paratyphi A infection.

General Safety assessments

Vaccination Phase

Vaccination with the GMO or placebo will take place on day -42 of the study and is repeated on day -32. Participants will be monitored for 60 minutes after vaccination to observe for unanticipated reactions.

Participants will be issued with an electronic diary (to allow remote monitoring) and an oral thermometer with instructions to record twice-daily temperatures, all adverse events, systemic symptoms and use of concomitant medication for 7 days after each vaccination. The scheduled follow-up visits during the vaccination phase are on days

-35, -32, -25, -18, and -7. At each visit safety will be assessed by review of diary card and interim medical history. Blood and stool samples will be collected for analysis at all follow-up visits; saliva will be collected at some visits.

Subjects will therefore be monitored closely following dosing with CVD 1902. If a subject is unwell or their electronic diary indicates that they are having symptoms or fevers, they can be reviewed by our study team and further samples can be taken for further cultures if felt to be clinically appropriate. If the participant is significantly unwell and needs further investigation or treatment (as decided by a medically qualified investigator) the participant will be referred for NHS care, either through the participant's GP or via the Infectious Diseases Consultant at the John Radcliffe Hospital, as appropriate. This will allow for a full range of appropriate investigations and management. Participants will not routinely be treated with antibiotics post vaccination but in the unlikely event that illness was felt to be related to infection with CVD 1902, the participant could be treated with antibiotics.

All subjects will, in any case, receive antibiotic treatment no later than 14 days following challenge with the wild type strain, as described below (under "***Antibiotic treatment***").

Challenge Phase

Challenge with the wild type *S.* Paratyphi A (NVGH308 strain) will take place on day 0 of the study. Participants will be issued with a diary card to record temperature and systemic symptoms daily for 14 days following challenge. They will also be issued with information on enteric precautions and study centre contact details. The scheduled follow-up visits during the challenge phase are daily from days 1 to 14. Each day, safety will be assessed by diary card review, measurement of vital signs, blood and stool sampling. Samples of blood, stool, and saliva will be collected for immunological, bacteriological and other analyses at specified timepoints.

Subjects will therefore be carefully monitored following challenge with the wild type strain for signs and symptoms of clinical infection with *S.* Paratyphi A. All subjects will receive antibiotic treatment at time of diagnosis or at 14 days following challenge with the wild type strain, whichever occurs sooner. To ensure antibiotic adherence, text message reminders will be sent when doses of antibiotics are due to be taken, unless the participant is due for a clinic visit, in which case the participant will be observed taking the dose at the visit.

Overall Assessments

Participants will have 24-hour phone access to a study physician from time of vaccination until the subject is deemed to be fully treated for S. Paratyphi A (see **Item 87**). Following challenge with the wild type *S.* Paratyphi A strain, participants will be encouraged to contact one of the study investigators on the 24-hour emergency mobile telephone number if they develop symptoms of paratyphoid

between the regular reviews. All participants will be required to carry a mobile phone which must be switched on at all times, throughout the vaccine and challenge periods until 21 days post challenge. All participants that have clinical paratyphoid fever, or have *S.* Paratyphi A isolated from their blood will be reviewed by a study doctor. The investigator will consider extra clinical review if the participants symptoms are moderate or severe. If participants are unwell as a result of *S.* Paratyphi A infection and are unable to attend the CCVTM for a visit, they may be visited at home by a study physician or asked to attend the John Radcliffe Hospital, Oxford. A phone call will be made to volunteers at day 21 post challenge to ensure they have completed their antibiotic course.

In addition, the study includes long-term follow-up visits at days 28, 90, 180 and 365 post challenge. Samples of blood and stool will also be collected at these points. Saliva will be collected at some of these visits.

Antibiotic treatment

Any volunteer diagnosed with paratyphoid fever following challenge (based on pre-defined clinical signs/symptoms and/or isolation of *S*. Paratyphi A from blood cultures) will be treated with antibiotics (oral ciprofloxacin 500mg twice daily for 7 days preferentially). This will lead to rapid clearance of the bacteria. The GMO has also been shown to be sensitive to ciprofloxacin.

All other volunteers who have not received a diagnosis of paratyphoid fever by day 14 post challenge will be treated with the same 7 day antibiotic course at that point.

To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

Control of the release

77. Methods and procedures to avoid and/or minimise the spread of the organisms beyond the site of release or the designated area for use.

Wild type *S.* Paratyphi A 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), advising 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, issuing 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),
- 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*. Paratyphi A 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.
- Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception 30 days prior to vaccination and until three negative stool samples have been obtained after completion of antibiotic treatment.
- 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.

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).

Participants will be screened for shedding of *S*. Paratyphi A in the stool. Stool cultures will be taken at Day-42 (first vaccination), D-35, D-32 (second vaccination), D-25, D-18, D-7 and at Day-0 (challenge), throughout the 14 day post-challenge period and at visits after paratyphoid diagnosis. Participants will be required to

supply 3 further stool samples until proven not to be shedding *S.* Paratyphi A in three consecutive samples. To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of 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.* Paratyphi A and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs).

The participants' GPs will also be notified of participation, vaccination, challenge and 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.

78. Methods and procedures to protect the site from intrusion by unauthorised individuals.

Entry to the facility is restricted to authorised trained personnel only (swipe card access and where applicable swipe card in combination with unique personal access codes). The activities at site are regularly monitored with onsite security service provisions established.

79. Methods and procedures to prevent other organisms from entering the site.

Where applicable personal protective equipment will be used and all site staff will follow local operating procedures for handling the GMO and infection control.

All GMO (and wild type) bacterial stocks are assessed for purity as part of the GMP manufacturing process. Good laboratory practice and aseptic technique when handling of the GMO will minimise the risk of other organisms entering the facility.

Waste treatment

80. Type of waste generated.

Clinical waste (including but not limited to: faecal/urine/blood/saliva/nasal swab samples, tissues, sharps, syringes, disposable clothing, gloves and aprons).

Laboratory waste (including but not limited to: plastic ware, microbiological waste (agar plates/blood culture bottles), gloves, disposable clothing, paper towels, and clinical samples as described above).

81. Expected amount of waste.

The amount of waste will be typical for the clinical site and laboratory operations amounting to a few clinical waste bags and bins per day waste. The amount of expected waste will be managed by standard operating procedures currently in place at the site.

82. Description of treatment envisaged.

All clinical and laboratory waste will be treated according to site standard operating procedures (SOPs) for handling GMO and potentially infectious GMO waste. All GMO activity at site will be inactivated by autoclaving prior to disposal and removal from the site. All associated procedures have been validated, site autoclaves are validated annually with contracts in place for regular equipment servicing and maintenance.

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

Emergency response plans

83. Methods and procedures for controlling the organisms 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*. Paratyphi NVGH308 strain.

Participants who vomit for any reason within 60 minutes of vaccination 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 disposed according to legal, local GMO and University of Oxford 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 vaccination period and until 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.* Paratyphi strain A (GMO and wild type NVGH308 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 (ciprofloxacin, azithromycin, or other macrolide antibiotics, co-trimoxazole, ceftriaxone) 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 planned. Participants will receive telephone calls or by text messages to remind them to take their antibiotic dose. They will record their doses of antibiotic in their electronic diary.

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

84. Methods, such as eradication of the organisms, for decontamination of the areas affected.

Contaminated areas may be decontaminated using Virkon or Haztab (granules or solution).

85. Methods for disposal or sanitation of plants, animals, soils and any other thing exposed during or after the spread.

All clinical and laboratory GMO waste will be autoclaved prior to removal and disposal at the site.

86. Methods for the isolation of the areas affected by the spread.

All participants will be monitored closely following each vaccination with the GMO via an electronic diary and follow up visits. They will be seen daily in the 14 days following challenge (if no diagnosis of paratyphoid fever has been made). All participants agree to have 24-hour contact with study staff during the vaccination period and until 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.

Participants will be screened for shedding of *S*. Paratyphi A in the stool. Stool cultures will be taken at Day-42 (first vaccination), D-35, D-32 (second vaccination), D-25, D-18, D-7 and Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after paratyphoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Paratyphi A in three consecutive samples. To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No instances of transmission to secondary contacts were detected in previous challenge studies conducted at the Oxford Vaccine Group.

The participant will provide letters from the study team to close contacts including household contacts. Contacts will be offered the opportunity to be screened for *S*. Paratyphi A infection, which will involve obtaining two stool samples 48-hours apart. If either stool culture of a household contact is positive, he/she will be referred to a Consultant in Infectious Diseases for appropriate antibiotic management and Public Health England will be informed.

87. Plans for protecting human health and the environment in case of the occurrence 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).

Participants will be screened for shedding of *S*. Paratyphi A in the stool. Stool cultures will be taken at Day-42 (first vaccination), D-35, D-32 (second vaccination), D-25, D-18, D-7 and Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after paratyphoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Paratyphi A in three consecutive samples. To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be

referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of 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 wild-type *S.* Paratyphi A and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants' GPs will also be notified of participation, vaccination, challenge and 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.

Part VI

A description of the methods used or a reference to standardised or internationally recognised methods used to compile the information required by this schedule, and the name of the body or bodies responsible for carrying out the studies.

The methods used in the application for Deliberate Release include the following:

- Construction of the GMO
- Stability and function of the GMO
- GMP manufacture of both the wild type and GMO *S*. Paratyphi CVD1902 strain.
- Experience of conducting controlled infection studies in healthy adult volunteers

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Date of document (day month year or month year)

APPLICATION FOR CONSENT TO RELEASE A GMO

PART A2: DATA OR RESULTS FROM ANY PREVIOUS RELEASES OF THE GMO

Give information on data or results from any previous releases of this GMO by you either inside or outside the European Community [especially the results of monitoring and the effectiveness of any risk management procedures].

The *S*. Paratyphi A CVD 1902 GMO strain has been generated and manufactured to GMP specifically for the purpose of serving as a live oral attenuated vaccine for use in the proposed controlled human infection study at the University of Oxford, according to previously established protocols. The GMO has been released previously in a single site phase I clinical trial conducted at the University of Maryland Center for Vaccine Development in the USA.

The GMO strain, CVD 1902 is derived from a wild type *S*. Paratyphi A 9150 strain. To date the Oxford Vaccine Group have conducted 2 paratyphoid challenge studies using the wild type *S*. Paratyphi NVGH308 strain:

1) A Salmonella Paratyphi A challenge study (REC ref. 14/SC/0004, OVG 2013/07)[9]

2) Investigating Enteric Fever – *Salmonella* Typhi and Paratyphi Challenge Study: (REC ref. 14/SC/1204, OVG 2014/01 NCT02192008 (active, recruitment complete).

PART A3: DETAILS OF PREVIOUS APPLICATIONS FOR RELEASE

Give details of any previous applications to release the GMO made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

The GMO *S*. Paratyphi A CVD 1902 strain has been generated and manufactured to GMP specifically for the purpose of undertaking the proposed controlled human infection study at the University of Oxford. No applications to release the GMO have previously been made to the Secretary of State under the 2002 Regulations or to another Member State under the Deliberate Release Directive 2001/18/EC.

PART A4: RISK ASSESSMENT AND A STATEMENT ON RISK EVALUATION

It is satisfied that the release of this GMO will not have an adverse effect on human health and the environment, providing the required precautionary measures are taken.

Risk Assessment: environmental impact of the release of the GMOs

This is the first proposal for the use of this GMO in a clinical study with healthy adult volunteers in the UK. 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 process. The wild type (parent strain) *S.* Paratyphi A has no known animal reservoir and does not persist in the environment at appreciable levels. The GMO has been shown to be no more persistent in the environment than the wild-type strain.

The GMO is intended for use as an attenuated oral vaccine agent in a controlled human infection model in healthy adult volunteers. The study participants will be randomized to receive vaccine or placebo. In those randomized to receive vaccine they will receive two doses of CVD 1902 orally. These participants 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 participants will undergo challenge with wild-type *S*. Paratyphi A strain. 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*. Paratyphi A in the stool. Stool cultures will be taken at Day-42 (first vaccination), D-35, D-32 (second vaccination), D-25, D-18, D-7 and 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 they showed no evidence of shedding *S.* Paratyphi A in three consecutive samples. To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No instances of transmission of *S*. Paratyphi NVGH308 strain 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 by email of the planned approximate initiation date for each study. Additionally they will be notified of those participants who receive challenge with wild-type *S.* Paratyphi A and those who have provided a full set of negative clearance samples. Additionally they will be informed of any participants who provide a positive clearance sample and the outcome of subsequent clearance samples. Any positive samples identified from screening of samples from contacts of participants will also be notified to PHE. Every effort will be made to obtain stool clearance samples, however PHE will be notified of any participant who fails to provide clearance samples.

Based on robust study procedures and completed studies to date, the challenge study is considered highly unlikely to result in wider public health concerns. Retention of a PHE challenge study database supports public health investigation and risk assessment. Public health action by PHE around the challenge study would be undertaken in a number of rare circumstances, such as:

- Statutory notification of clinical or microbiologically-confirmed enteric fever in a close contact (e.g. household contact) of a challenge study participation.
- Microbiological notification of a challenge strain of *S.* Paratyphi A being identified at the national reference laboratory in public health testing. This may include relapse of study participants or disease episodes in people not known to be participating in the studies. The database will support identification of any “false positive” notifications in the unlikely event of challenge participants being tested or reported through NHS channels into national reference microbiology.
- Review of the PHE challenge study database and additional microbiology may be undertaken in the event of enteric fever cases resident in or with connection to Oxfordshire who do not have a relevant travel history or epidemiological connection to an enteric fever case with travel history.

Additional to routine public health response informed by national guidance, PHE will notify the OVG challenge study team in the event of apparent onward transmission from study participants, to enable further investigation and any necessary research governance activities.

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.* Paratyphi A and the closely related species *S.* Typhi are highly monomorphic, meaning there is very little genetic variation within the global *S.* Typhi/Paratyphi A population, thereby indicating that its propensity for genetic exchange is extremely low. This is supported by a study that analysed whole genomes of 19 *S.* Typhi strains and identified only 1954 single nucleotide polymorphisms (SNPs) between all of them; approximately 1 every 2300bp[11].

Genomic insertions were rare in the sequenced isolates and evolution in the *S.* Typhi/Paratyphi A 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 typhoidal serovars 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 *guaBA* operon *and clpX* gene to regain a wild type 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 the GMO strains lacking the *guaBA* operon *and clpX* gene have reduced virulence. For the safety of our study participants their health will be monitored very closely by our clinical study team.

Risk assessment: factors affecting dissemination

Dispersal of wild type *S.* Paratyphi A occurs via faeco-oral transmission (contamination of food or water with faeces of infected individuals). There is a short survival time for wild type *S.* Paratyphi A in chlorinated drinking water. The GMO does not have a selective or survival advantage over wild-type *S.* Paratyphi A and will not persist in the environment.

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. A GMO *S.* Typhi strain has been used previously in clinical studies with healthy adult volunteers by the Oxford Vaccine Group (DEFRA Refs 16/R48/01 and 18/R48/01). During these studies, no release of the GMO beyond the normal sewage and water treatment processes was detected or suspected. It is expected, based on evaluation of shedding in the phase 1 clinical trial, that the GMO will be shed by volunteers for approximately 3 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 faecal-oral transmission.

Risk assessment: human health impact

The GMO has been released previously in a single site phase I clinical trial conducted at the University of Maryland Center for Vaccine Development in the USA. This study showed that the GMO was safe and well tolerated.

In the proposed study described in this application, clinical study participants will be randomized to receive two doses of either the GMO or placebo. They will then be monitored for signs of infection with the GMO although based on the results of the initial phase 1 study this is considered to be highly unlikely. The participants will be monitored remotely via an electronic diary and at regular clinical visits. They will not be treated with antibiotics routinely during the vaccination phase. Although the GMO has confirmed sensitivity against first, second, third and fourth line antimicrobials, clearance of the GMO is expected to occur without causing infection and without the need for antibiotics. Participants have 24 hour telephone access to a study doctor throughout the study.

The GMO 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).

Following receipt of two doses of vaccine or placebo all participants will be challenged with wild-type *S*. Paratyphi A. Participants will then be seen daily in clinic for up to 14 days. Safety bloods and blood and stool cultures will be monitored closely during this time. All participants are then treated with a 7 day course of antibiotics either at the time of paratyphoid diagnosis or at day 14 post challenge, whichever is sooner.

Participants will be screened for shedding of *S*. Paratyphi A in stool samples following vaccination. Stool cultures will be taken at Day-42 (first vaccination), D-35, D-32 (second vaccination), D-25, D-18, D-7 and Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after paratyphoid diagnosis. Participants will also be required to supply further stool samples until proven not to be shedding *S.* Paratyphi A in three consecutive samples. To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No instances of transmission of the challenge strains of *S*. Typhi or *S.* Paratyphi A to secondary contacts were detected in previous challenge studies conducted at the Oxford Vaccine Group.

Strict exclusion criteria have been set for the study including criteria to minimize the risk of transmission of the GMO to vulnerable groups. 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*. Paratyphi A 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. Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception 30 days prior to challenge and until three negative stool samples have been obtained after completion of antibiotic treatment. 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.

Risk assessment: environmental impact

S. Paratyphi A 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, providing the participant with stool sampling equipment, education of participants on correct hand washing techniques (including demonstration and observation), advising 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, issuing 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*. Paratyphi A 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. Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception 30 days prior to challenge and until three negative stool samples have been obtained after completion of antibiotic treatment. 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.

All site GMO waste will be disposed according to local GMO standard operating procedures.

Risk assessment: monitoring the GMO

Stool cultures will be taken at Day-42 (first vaccination), D-35, D-32 (second vaccination), D-25, D-18, D-7 and Day 0 (challenge), daily throughout the 14 day post-challenge period and at visits after paratyphoid diagnosis. Study participants could potentially still be shedding GMO throughout this time period, although this is very unlikely. Additionally, quantitative stool cultures or PCR may be performed to assess the burden of stool shedding. Isolates from stool samples may be stored frozen for future analysis, which may include phage typing or genetic sequencing.

To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

Blood samples will be monitored at visits during the vaccination period and daily post challenge for *S.* Paratyphi A using a combination of microbiological techniques. Due to CVD 1902 being a guanine auxotroph, culture of the GMO vaccine strain requires guanine supplementation of media. Automated BACTEC 9240 continuous monitoring system will be used to culture organisms which will be identified as *S*. Paratyphi A via biochemical and serological methods. Confirmed isolates will be tested for antibiotic susceptibility using standard methods. Positive isolates obtained during the vaccination period will be assumed to be due to the vaccine strain. However should this need arise, deletion of the *guaBA* operon and *clpX* gene can be readily identified by specific methods such as PCR or nucleotide sequencing.

Participants will be issued with an electronic diary (to allow remote monitoring) and an oral thermometer with instructions to record twice-daily temperatures, all adverse events, systemic symptoms and use of concomitant medication for 7 days after each vaccination. The scheduled follow-up visits during the vaccination phase are on days -35, -32, -25, -18, and -7. At each visit safety will be assessed by review of diary card and interim medical history. Blood and stool samples will be collected for analysis at all follow-up visits. Following challenge with wild-type *S*. Paratyphi A participants are

reviewed in clinic daily for the next 14 days. Participants are also required to complete twice-daily temperatures and an electronic diary of symptoms and medications for 21 days post challenge. The protocol for visits will depend on whether the participant develops infection following challenge with the wild type *S.* Paratyphi or not. Following diagnosis of enteric fever blood and stool sampling with be performed at 12, 24, 48, 72 and 96 hours post diagnosis. Following completion of antibiotic treatment and confirmed clearance of *S.* Paratyphi A in stool samples participants will be monitored at long term follow-up visits at Day 28, 90, 180 and 365. All participants agree to have 24-hour contact with study staff during the vaccination period and until 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 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*. Paratyphi A 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.

<u>Risk assessment: emergency response</u>

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).

Participants will be screened for shedding of *S*. Paratyphi A in the stool. Stool cultures will be taken at Day-42 (first vaccination), D-35, D-32 (second vaccination), D-25, D-18, D-7 and Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. To detect chronic carriage of *S*. Paratyphi A, 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.* Paratyphi A infection and no longer an infection risk. If a clearance sample(s) is found to be positive a further course of antibiotics will be given. If samples remain

positive for *S.* Paratyphi A four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of transmission of the wild type *S*. Paratyphi NVGH308 strain 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.* Paratyphi A, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants' GPs will also be notified of participation, vaccination, challenge and 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.

Participants who vomit for any reason within 60 minutes of the GMO vaccination 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 disposed 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 vaccine. All participants agree to have 24-hour contact with study staff following first vaccination until four weeks post challenge 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.* Paratyphi strain (GMO and wild type NVGH308 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 (ciprofloxacin, azithromycin or other macrolide antibiotics, co-trimoxazole or ceftriaxone) 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 (or other suitable inpatient ward at John Radcliffe Hospital, Oxford) in cases of severe paratyphoid fever and/or other circumstances.

PART A5: ASSESSMENT OF COMMERCIAL OR CONFIDENTIALITY OF INFORMATION CONTAINED IN THIS APPLICATION.

Identify clearly any information that is considered to be commercially confidential. A clear justification for keeping information confidential must be given.

It is our assessment that there is no commercially sensitive information contained within this application. The information contained herein pertains to a novel strain of *Salmonella enterica* subsp. *enterica* serovar Paratyphi A engineered to be growth deficient. The strain will be used as a live oral attenuated vaccine in a controlled human infection study to be performed at the Oxford Vaccine Group (University of Oxford), Churchill Hospital, Oxford. The trial protocols will be registered on publically available databases, including clinicaltrials.gov and the European Clinical Trials Database, following receipt of the necessary approvals. This study is sponsored by the University of Oxford and supported by a grant from the Medical Research Council.

PART A6: STATEMENT ON WHETHER DETAILED INFORMATION ON THE DESCRIPTION OF THE GMO AND THE PURPOSE OF RELEASE HAS BEEN PUBLISHED

Make a clear statement on whether a detailed description of the GMO and the purpose of the release have been published, and the bibliographic reference for any information so published.
This is intended to assist with the protection of the applicant's intellectual property rights, which may be affected by the prior publication of certain detailed information, e.g. by its inclusion on the public register.

There are currently no publications providing a detailed description of the specific GMO (*S.* Paratyphi CVD 1902 strain) though it is referenced in a single paper detailing immune response[8]. This strain has been constructed by the Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, USA specifically for use as a vaccine against *Salmonella* Paratyphi A. It is being manufactured by Bharat Biotech International specifically for use in a human infection model at the Oxford Vaccine Group, University of Oxford.