PART A1: INFORMATION REQUIRED UNDER ARTICLE 11 (SCHEDULE 1) OF THE 2002 REGULATIONS.

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.

Name and Address of the Applicant:

Emergent Europe Ltd,
540-545 Eskdale Road,
Winnersh Triangle,
Wokingham,
Berkshire, RG41 5TU
United Kingdom

Emergent Europe Ltd was formerly Microscience Ltd. The company name changed in July 2005.

2. The title of the project.

A double blind, placebo controlled study to evaluate the safety and immunogenicity of escalating doses of a candidate oral immunotherapy (M04NM11, formerly called hepatitis B Candidate 1) in patients who have chronic Hepatitis B infection.
Part II

Information relating to the organisms

Characteristics of the donor, parental and recipient organisms

3. Scientific name and taxonomy.

This information is provided in the equivalent section of the previously approved application for deliberate release of this GMO, reference 02/R37/2.

Refer to Item 32 of this current application for further details of the previous application and consent for deliberate release of this GMO.

Note:
Gene A referred to throughout the previous application and this application is gene aroC.
Gene B referred to throughout the previous application and this application is gene ssaV.

4. Usual strain, cultivar or other name.

This information is provided in the equivalent section of the previous application 02/R37/2.

5. Phenotypic and genetic markers.

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

7. The description of identification and detection techniques.

PCR may be used to detect the parent and recipient organisms. Information relating to the PCR technique is given in the equivalent section of the previous application 02/R37/2.

Laboratory culture of the GMO is used routinely as a detection method (see appendix 1 in the confidential annex).
8. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

This information is provided in the equivalent section of the previous application 02/R37/2.

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.

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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.

The classification of hazard relates to the parent strain S. typhi. The key legislation controlling notification, quarantine, exclusion of food handlers, etc are:

- Genetically modified organisms (Deliberate Release) Regulations 2002

S. typhi is covered under this legislation as the cause of typhoid fever. S typhi is covered by the Terrorism Act, as well as the ACDP regulations:

- Control of Substances Hazardous To Health Regulations (2002)
- Categorisation of biological agents according to hazard and categories of containment (Second Supplement to fourth edition 1995) (2000)
- ACDP Approved List of Biological Agents. Published by HSE, 2004.
(b) **the generation time in natural ecosystems, and sexual and asexual reproductive cycle:**

This information is provided in the equivalent section of the previous application 02/R37/2.

(c) **survivability, including seasonability and the ability to form survival structures, including seeds, spores and sclerotia:**

This information is provided in the equivalent section of the previous application 02/R37/2.

(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:**

This information is provided in the equivalent section of the previous application 02/R37/2.

(e) **antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy:**

This information is provided in the equivalent section of the previous application 02/R37/2.

(f) **involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration:**

This information is provided in the equivalent section of the previous application 02/R37/2.

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.**

This information is provided in the equivalent section of the previous application 02/R37/2.

14. **The history of previous genetic modifications.**

This information is provided in the equivalent section of the previous application 02/R37/2.

**Characteristics of the vector**

15. **The nature and source of the vector.**

This information is provided in the equivalent section of the previous application 02/R37/2.
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.

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

Characteristics of the modified organisms

19. The methods used for the modification.

This information is provided in the equivalent section of the previous application 02/R37/2.

20. The methods used.

(a) to construct the insert or inserts and introduce them into the recipient organism

This information is provided in the equivalent section of the previous application 02/R37/2.

(b) to delete a sequence

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.
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.

This information is provided in the equivalent section of the previous application 02/R37/2.

23. The methods and criteria used for selection.

This information is provided in the equivalent section of the previous application 02/R37/2.

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.

This information is provided in the equivalent section of the previous application 02/R37/2.

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.

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

27. The stability of the organism in terms of genetic traits.

This information is provided in the equivalent section of the previous application 02/R37/2.

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.

The antigen is expressed by the GMO under the control of an inducible in vivo regulated promoter. Therefore, expression of the antigen by the GMO is induced under intracellular conditions.
Approximately 0.5 µg/mL antigen is expressed by 1 x 10^9 Colony Forming Units (CFU)/mL GMO (as quantified by ELISA), after overnight survival in a human macrophage-like cell line (U-937 cells) (see appendix 2 in the confidential annex).

29. The activity of the gene product.
This information is provided in the equivalent section of the previous application 02/R37/2.

30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector.
This information is provided in the equivalent section of the previous application 02/R37/2.

31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.
This information is provided in the equivalent section of the previous application 02/R37/2.

32. The history of previous releases or uses of the organisms.
The GMO has previously been released, in accordance with the conditions applied to the consent for deliberate release of the GMO (application reference number 02/R37/2; application made by Microscience Ltd, company name change to Emergent Europe Ltd, July 2005). Consent was notified on 16 July 2003.

The GMO was released following oral administration to 30 healthy adult volunteers in a Phase I clinical study designed to assess safety and immunogenicity of this potential immunotherapy. In this study the volunteers shed small quantities of the GMO in their faeces and this constituted the release of the GMO. The release was into the Greater London sewage system.

The design of the Phase I clinical study was such that 30 subjects were to receive 2 doses of the GMO given 56 days apart; 10 subjects receiving 2 doses of 1 x 10^8 CFU (low dose group) and 20 subjects receiving 2 doses of 1 x 10^9 CFU (high dose group). The GMO was administered orally in a volume of sodium bicarbonate solution. Safety monitoring was a critical component of the study and therefore the vaccinees were closely monitored for any signs of infection with the GMO and for the presence of the organism in blood, faeces and urine.

- Subjects were kept at the clinical site for 6 hours immediately after vaccination and evaluated carefully for evidence of fever, chills, headache, abdominal pain or abdominal cramping. Subjects then returned to the study site daily for the next 7 days and then on days 11, 14, 21 and 28 after vaccination.
Subjects completed diary cards throughout the 28 day period following each vaccination to note their temperature twice daily and the occurrence of any symptoms including loss of energy, chills, headache, muscle or joint aches, loss of appetite, nausea, vomiting, gas, constipation, abdominal cramps or pain. The subjects were to contact the investigative site if they developed pre-defined symptoms suggestive of a potential clinical infection with the vaccine strain. Subjects were able to return to the study site for unscheduled visits at any time if they were at all concerned.

Blood samples were collected on days 4, 7, 11, 14 and 28 after vaccination and cultured for the presence of *Salmonella* using routine techniques by the hospital pathology service.

Stool samples were collected on days 1, 2, 3, 4, 5, 6, 7, 11, 14 and 28 after vaccination and cultured for the presence of *Salmonella* using routine techniques as described in Item 73.

Urine was collected on days 4, 7, 11, 14, 21 and 28 after vaccination and tested by dipstick for leukocyte esterase and nitrites; If the dipstick was positive for both leukocyte esterase and nitrites a fresh sample was sent for culture and antibiotic sensitivity testing.

The protocol specified that if at any time a subject was considered to have a clinically-significant infection with the GMO they would be treated with antibiotics and followed up until the infection was clear. A subject was to be considered infected if they displayed any of the following signs or symptoms:

- The investigator considered the subject to be infected.
- Two blood cultures were positive for the GMO, even if the subject did not display any signs or symptoms of infection.
- Symptomatic bacteraemias attributable to the GMO.
- Any subject demonstrated persistent (beyond day 7) faecal shedding of the GMO.

For an infection to have been considered clear all blood, stool and urine samples would have to be negative for the GMO on two consecutive days following the course of antibiotic therapy.

The immunotherapy was administered to the volunteers at BIBRA International Ltd, Carshalton, Surrey, UK. The first subjects were dosed on 27 August 2003 and the last day of dosing was 15 January 2004. 10 subjects received 2 doses of $1 \times 10^8$ CFU, 18 subjects received 2 doses of $1 \times 10^9$ CFU and 2 subjects received a single dose of $1 \times 10^9$ CFU. The vaccine was well tolerated by all subjects, there were no unexpected adverse events and no serious adverse events related to the investigational product reported.

The subjects were closely monitored for presence of the GMO in blood and urine and the GMO was not isolated from any of the blood or urine samples. Shedding of the GMO in the faeces of vaccinated subjects was closely monitored by culturing stool samples for *Salmonella* on frequent specified occasions throughout the duration of the trial. In line with expectations, there was no persistent shedding and shedding did not occur in any subject beyond 6 days after dosing.
Following administration of the first dose of immunotherapy there was little difference in faecal shedding profiles of subjects in the high (1 x 10⁹ CFU) and low dose (1 x 10⁸ CFU) groups and shedding did not occur in any subject beyond Day 5. Twenty of the 30 subjects shed *S. typhi* for at least one day with the majority shedding over the first 3 days only.

- Four of the 10 subjects in the low dose group shed the GMO; 3 subjects shed over the first 3 days only, 1 subject shed on Day 4.
- Sixteen of the 20 subjects in the high dose group shed the GMO; the majority of subjects did not shed beyond 3 days after dosing, 2 subjects shed over the first 4 days and 2 subjects shed over the first 5 days.

Following the second dose the faecal shedding profiles were similar between the 2 dose groups and similar to the profiles around the first dose. Shedding did not occur in any subject beyond Day 6. Twenty-two of the 28 subjects shed *S. typhi* for at least one day with the majority shedding over the first 3 days only.

- Seven of the 10 subjects in the low dose group shed the GMO, none beyond Day 3.
- Fifteen of the 18 subjects in the high dose group shed the GMO: 14 subjects shed over the first 3 days only and 1 subject shed over the first 4 days.

Therefore over the course of the study 20 doses of 1 x 10⁸ CFU and 38 doses of 1 x 10⁹ CFU of the immunotherapy were administered and no shedding was detected beyond 6 days after dosing (refer to appendix 3 of the confidential annex). The release of the GMO into the Greater London sewage system therefore occurred between the dates 27 August 2003 and 22 January 2004. As replication of the GMO can not occur in the host, the maximum number of GMO organisms shed was 20 doses of 1 x 10⁸ CFU plus 38 doses of 1 x 10⁹ CFU; approximately 4 x 10¹⁰ CFU.

The shedding profile observed for the GMO was similar to the profile previously observed for the recipient strain and a related *S. typhi* strain (refer to Item 33(b) and Item 54 for details of clinical studies performed to assess safety of the recipient and a related Emergent Europe Ltd vaccine strain respectively).

The shedding profile of the GMO contrasts greatly with the pattern resulting from infection with wild type *S. typhi*, and this is due to the severe attenuation of the GMO. In a proportion of infected individuals, wild type *S. typhi* is able to colonise the gall bladder where the cells replicate and then enter the bile which carries the organism to the GI tract; from here they are shed in faeces. Long term shedding with wild type *S. typhi* is therefore a result of gall bladder infection which in turn occurs as a result of the organisms being widely disseminated during the bacteraemic phase of an infection. As the GMO is severely attenuated and can not give rise to systemic infections and bacteraemias, it can not colonise the gall bladder, or anywhere else in the digestive tract. Shedding of the GMO in faeces therefore only occurs as a result of the organisms passing directly through the GI tract following ingestion, and lasts only for the time it takes for GI transit to be completed.
33. In relation to human health, animal health and plant health

In order to support the safety of the GMO in the planned clinical development programme, Emergent Europe has conducted extensive pre-clinical studies. These data formed part of the application (CTX) to the Medicines Control Agency (MCA) from whom approval was obtained prior to the start of the previous clinical trial. An expanded pre-clinical data set, combined with the clinical data now available, form part of the application to the Medicines and Healthcare products Regulatory Agency (MHRA) from whom approval will be obtained prior to the start of the proposed clinical trial.

(a) the toxic or allergenic effects of the organisms and/or their metabolic products:

The GMO has previously been administered to 30 healthy subjects in a Phase I clinical trial and no toxic effects were reported (refer to Item 32). The Hepatitis B antigen expressed \textit{in vivo} by the GMO has also been administered to humans in a separate study with no toxic effects (see appendix 37 in confidential annex of previous application 02/R37/2).

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

Wild type \textit{S. typhi} and thus the recipient and GMO do not infect animals or plants as humans are the only hosts and vectors for \textit{S. typhi}.

Regarding pathogenicity in humans, the wild type parent strain, \textit{S. typhi} Ty2 from which the recipient and GMO were derived, was originally isolated from an infected patient, and is virulent in humans causing the typical symptoms of typhoid disease. The GMO and the recipient \textit{S. typhi} strain are severely attenuated through deletions in both gene A and gene B. Both strains have been assessed for safety in Phase I clinical studies involving healthy adults and both strains demonstrated a comparable safety profile, being well tolerated and safe in all subjects. The findings of these studies therefore show that the recipient strain and the GMO are non-virulent in healthy adult human subjects. The GMO was assessed in a Phase I study involving 30 healthy volunteers who received up to 2 doses of up to $1 \times 10^9$ CFU. The recipient strain has been assessed in three clinical trials involving a total of 89 healthy subjects who received up to $5 \times 10^9$ CFU. In all studies safety monitoring was a critical component and included monitoring the vaccinees closely for any signs or symptoms of infection related to the attenuated \textit{S. typhi} strains. Subjects were monitored for the presence of the organism in blood and urine, and stool samples were cultured regularly to establish the shedding profile in faeces and to determine whether there was persistent shedding (defined as shedding beyond day 7) which would be a sign that the organism had been able to establish a systemic infection leading to release of the organism into the digestive tract. As described in more detail in Item 32 shedding of the recipient and GMO was expected to be, and is indeed, transient as in contrast to wild type \textit{S. typhi} which, following a systemic infection, is able to colonise the gall bladder and from there shed into faeces, the attenuated recipient and GMO strains can not colonise or replicate in the host. Therefore shedding occurs only for as long as it takes for the organisms to pass directly through the GI tract after administration.
Details of the clinical trials demonstrating safety of the GMO and recipient are given below.

**Clinical trial performed to assess the safety and immunogenicity of the GMO**
The GMO has been administered to 30 healthy adult volunteers receiving up to 2 doses of up to $1 \times 10^9$ CFU (10 subjects received 2 doses of $1 \times 10^8$ CFU, 18 subjects received 2 doses of $1 \times 10^9$ CFU, and 2 subjects received one dose of $1 \times 10^9$ CFU). The GMO was well tolerated with a good safety profile; there were no unexpected adverse events, no bacteraemias and shedding of the strain in the stool of subjects was not beyond 6 days for any subject, at any dose level. Detail of this clinical study is provided in Item 32.

**Clinical trials performed to assess the safety and immunogenicity of the recipient strain**
The first study (an Investigator sponsored study, Principal Investigator DJM Lewis, Hindle et al. 2002, copy of publication provided as appendix 13 in confidential annex of previous application 02/R37/2) was a dose escalation study involving three groups of 3 subjects vaccinated with $10^7$, $10^8$, or $10^9$ CFU of the recipient strain administered orally in bicarbonate solution. Volunteers were resident in the Vaccine Institute (St George’s Vaccine Institute, London, UK) for 72 hours post administration to enable isolation and careful observation, and were followed up for safety assessments daily for one week and weekly for 28 days. Safety assessments included monitoring blood, stool and midstream urine cultures for the recipient strain (on the day of administration, daily for the first seven days and weekly for 28 days after administration). The vaccine was found to be safe and well tolerated in all subjects. Blood and urine cultures were negative for all subjects. Shedding of the recipient strain was detected in only one of the 3 subjects in each group. This occurred soon after vaccination and lasted 1 day only, except for 1 subject receiving $10^9$ organisms, who shed on Days 1, 3, and 7.

The second study (Kirkpatrick et al. 2006, a copy of the publication is provided as appendix 4 in the confidential annex) was a placebo controlled, double blind, single oral dose, dose escalation study, to determine the safety and immunogenicity of three dose levels ($5 \times 10^7$ CFU, $5 \times 10^8$ CFU, $5 \times 10^9$ CFU) of the recipient vaccine strain. In this study 60 healthy adult subjects received one of three dose levels of vaccine (n=16) or placebo (n=12) and were followed up frequently for 28 days following dosing, then at 3 months and 6 months. The study was an outpatient study performed at two centres in the US under a Company sponsored IND. Safety monitoring was of prime concern in this study and the strategy is outlined below:

- Subjects remained at the study site for at least 90 minutes after dosing and were evaluated for any signs of adverse events. Subjects then returned to the study site daily for the next 7 days and then on study days 10, 12, 14, 21 and 29 plus months 3 and 6.
- Subjects completed diary cards throughout the 28 day study period to note their temperature twice daily and the occurrence of any symptoms including loss of energy, chills, headache, muscle or joint aches, loss of appetite, nausea, vomiting, gas, constipation, abdominal cramps or pain. Subjects were able to return to the study site for unscheduled visits at any time if they are at all concerned.
• Blood cultures were collected daily for the first 7 days after vaccination and then at
days 10, 12, 14, 21 and 28; and at months 3 and 6.
• Stool samples were collected over every 24 hour period for the first 7 days after
vaccination and then at days 10, 12, 14, 21 and 28. Samples were also obtained at
month 3 for all subjects and at month 6 for subjects who received active medication.
• Urine was collected daily for the first 7 days after vaccination and then at days 14, 21
and 28; and at months 3 and 6. Samples were tested by dipstick for leukocyte
esterase and nitrites and if the dipstick was positive for either, a sample was sent for
culture and antibiotic sensitivity testing.
• According to the study design if at any time a subject was considered to have been
clinically infected with the vaccine strain they would have been treated at the
Investigator’s discretion with antibiotics and followed-up until the infection was clear.
For the infection to be considered clear all blood, stool and urine samples would
have to have been negative for the vaccine strain on two consecutive days following
the course of antibiotic therapy.

Findings showed that the vaccine was well tolerated by all the subjects dosed, no serious
adverse events related to study medication were reported and there were no unexpected
adverse events. There was no significant difference between the adverse event profile in
subjects given placebo or vaccine. No bacteraemias were reported and none of the urine
cultures were positive for the recipient strain. Persistent shedding in faeces (> 7 days) was
not observed in any of the volunteers in any of the 3 groups. Twenty-six of the 48 subjects
who received the recipient strain shed S. typhi in their faecal stools on at least one day, and
the number shedding appeared to increase with increasing dose level (see appendix 3 in
the confidential annex). However, most of the shedding occurred in the first 3 days and no
subject shed beyond day 6.
• Four of the 16 subjects in the 5 x 10^7 dose group shed the recipient strain, none
sheding beyond Day 3 post-vaccination.
• Nine of the 16 subjects in the 5 x 10^8 dose group shed the recipient strain, none
sheding beyond Day 3 post-vaccination.
• Thirteen of the 16 subjects in the 5 x 10^9 dose group shed the recipient strain. Ten
subjects shed for up to 3 days following vaccination, 1 subject shed to Day 4, 1
subject shed to Day 5 and 1 subject shed to Day 6.

The recipient strain was not isolated from any of the stool samples cultured 3 and 6 months
following dosing, indicating the subjects had not become chronic carriers of the organism.

The third study (Kirkpatrick et al. 2005, a copy of the publication is provided as appendix 5
in the confidential annex) was a single-centre open label, randomised, parallel group study
designed to determine the safety and immunogenicity of two different presentations of 5 x
10^9 CFU of the recipient strain administered orally as a single dose. This outpatient study
was performed in the US under a Company sponsored IND. Again, in this study the vaccine
was found to be safe and well tolerated. None of the subjects developed a bacteraemia or
persistent faecal shedding of the vaccine strain (> day 7). A total of 16 healthy volunteers
received presentation 1 and 16 received presentation 2, both comprising vaccine in
bicarbonate solution. Safety assessments were as follows:
Subjects remained at the study site for at least 90 minutes post dosing and were evaluated for any signs of adverse events. Subjects then returned to the study site daily for the next 7 days and then on study days 10, 14, 21 and 28.

Subjects completed diary cards for 14 days following administration to note their temperature twice daily and the occurrence of any symptoms including chills, headache, abdominal discomfort, abdominal cramping, feeling feverish. The subjects were to contact the investigative site if they felt feverish. Subjects were able to return to the study site for unscheduled visits at any time if they are at all concerned.

Stool samples were collected on days 7 and 10 for culturing, to detect if any subject persistently shed the vaccine in their stools (> 7 days). Additional stool samples were to be collected if the subject developed a fever or at other times considered necessary by the Investigator to screen for a clinical infection due to the vaccine strain. No persist shedding was observed; all stool samples were negative for the vaccine strain.

Blood samples were to be collected if the subject developed a fever or at other times considered necessary by the Investigator to screen for a bacteraemia. All blood samples were negative for the vaccine strain.

Urine was collected on day 7 and tested by dipstick for leukocyte esterase and nitrites. If the dipstick was positive for either, a sample was sent for culture and antibiotic sensitivity testing. Additional urine samples were to be collected if the subject developed a fever or at other times considered necessary by the Investigator to screen for a clinical infection due to the vaccine strain. All urine samples were negative for the vaccine strain.

According to the study design if at any time a subject was considered to have been clinically infected with the vaccine strain they would have been treated at the Investigator’s discretion with antibiotics and followed-up until the infection was clear. For the infection to be considered clear all blood, stool and urine samples would have to have been negative for the vaccine strain on two consecutive days following the course of antibiotic therapy. Antibiotic treatment related to vaccine treatment was not required for any subject.

In summary, a total of 89 healthy adult subjects have been vaccinated with the recipient strain. The vaccine demonstrated a good safety profile and was well-tolerated by all subjects. No vaccine-related serious adverse events were reported. No bacteraemias were reported. Shedding in stool did not occur in any subject beyond 7 days after vaccine administration and the majority of shedding occurred within the first 3 days after dosing.

References
Kirkpatrick et al. 2006. Vaccine  24(2) 116-123
(c) the capacity of the organisms for colonisation:

The wild type strain is severely host restricted, only having the capacity to colonise humans. The recipient strain and the GMO are severely attenuated and are unable to colonise humans because of their inability to replicate in the host.

(d) if the organisms are pathogenic to humans who are immunocompetent

(i) diseases caused and mechanisms of pathogenicity including invasiveness and virulence
(ii) communicability
(iii) infective dose
(iv) host range and possibility of alteration
(v) possibility of survival outside of human host
(vi) presence of vectors or means of dissemination
(vii) biological stability
(viii) antibiotic-resistance patterns
(ix) allergenicity
(x) availability of appropriate therapies.

The GMO is not expected to be pathogenic in immunocompetent subjects. Moreover, the recipient strain has shown to be completely safe in immuno-compromised models, particularly those relevant to Salmonella infection (see appendix 24 in the confidential annex of previously approved application 02/R37/2).

(e) the other product hazards:

No hazards are anticipated to result from release of the GMO due to the severe attenuation of this strain and the non-toxicity of the expressed antigen.
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 organism as or in a product in the future.

The GMO will be administered to patients with chronic Hepatitis B in a Phase II placebo-controlled clinical study. The clinical protocol (see appendix 6 in the confidential annex) will be submitted to the Medicines and Healthcare products Regulatory Agency (MHRA) as part of a clinical trials application (CTA). The purpose of the study is to determine the safety and immunogenicity of this potential immunotherapy after oral administration to the patients. In relation to the release of the GMO, the main points are summarised below.

Subjects will be recruited at 2 clinical study sites in London:
Site 1 – Clinical Research Centre, Royal London Hospital, London E1 2AT (CRC).
Site 2 - King’s College London School of Medicine, London SE5 9PJ (KCL).

It is currently anticipated that subject numbers will be split approximately evenly between the two sites.

The placebo and GMO will be diluted in sodium bicarbonate solution for oral administration. Fifteen volunteers will receive placebo and 30 volunteers will receive up to $10^{10}$ CFU of the GMO on six occasions, 28 days apart. Following administration the GMO may be transiently shed in the faeces of subjects but this is not expected to last beyond 7 days after dosing.

Shedding in faeces thus constitutes the release of the GMO and consequently the GMO may be released into the sewage system. Release will be restricted to England; as a condition of the protocol, the volunteers will be requested not to leave England for the first 14 days following dosing. Subjects will reside within the Greater London area and so the majority of any released organisms will enter the Greater London sewage system.

In relation to release into the sewage system Emergent Europe has generated data that shows the GMO does not survive in raw sewage (refer to Item 54 for details). In addition, stools from any patient suffering from wild type typhoid infection are discharged into the sewage system and they are effectively contained and inactivated by normal sewage treatment processes; the UK sewage system has significantly contributed to the eradication of enteric diseases such as typhoid and cholera from the population. Any GMOs, which enter the sewage system, do have the potential to come into contact with the environment (e.g. soil and water bodies), however wild type S. typhi is known not to persist in the environment and all available data indicates that the attenuated Emergent Europe GMO which is a highly weakened form of S. typhi will also not persist in the environment (refer to Item 55 for details of supporting data).
The shedding profile of the GMO in faeces was determined in a Phase I study which is described in Item 32. The data showed that the majority of shedding occurred within the first 3 days after administration of the GMO and did not occur beyond 6 days after dosing. Comparable shedding profiles are displayed by the recipient strain (refer to Item 33(b)) and a related Emergent Europe S. typhi vaccine strain that has the same mutations in gene A and gene B (refer to Item 54). The shedding profile of these severely attenuated S. typhi strains (transient shedding, not beyond day 7) contrasts with long term shedding sometimes displayed by patients infected with wild type S. typhi and is due to the inability of the GMO to multiply within or colonise the host. In a proportion of individuals infected with wild type S. typhi the systemic phase of the infection leads to colonisation of the gall bladder where the cells replicate and then enter the faeces of the host in bile. As the GMO can not give rise to systemic infections it can not colonise the digestive tract, and so shedding of the GMO can only occur transiently, for as long as it takes for the organisms to pass directly through the GI tract following ingestion.

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 will be stressed. Faecal-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. Women who are breast-feeding and individuals who work as commercial food handlers are excluded as are individuals who are health care workers with direct contact with high-risk patients, child care workers or other individuals who have routine contact with children less than 2 years of age. Individuals with household contact with immuno-compromised individuals, pregnant women or the young and elderly will also be excluded from the trial.

With regard to the dosing schedule for the study, for the first dose each subject assigned to the active group (as opposed to the placebo group) will receive $10^6$ CFU of the GMO and if this dose is tolerated the subject will receive a dose of $10^8$ CFU 28 days after the first dose. If this second dose is tolerated they will receive a dose of $10^{10}$ CFU 28 days after the second dose. If the $10^{10}$ CFU dose is tolerated they will receive a further 3 doses of $10^{10}$ CFU at 28 day intervals with the objective of delivering a total of 6 doses. If subjects do not tolerate a dose increase, future planned doses may be reduced to a previously tolerated dose level or they may be missed. Further detail of the study protocol is given in Item 76.

If the success criteria are met in the proposed clinical study then the GMO may form the basis of a product in the future.
35. The intended dates of the release and time planning of the experiment including frequency and duration of releases.

The clinical study will begin in May 2006, the first subject being dosed within 2 to 4 weeks of study start. The duration of the study will depend on the rate of recruitment. Recruitment is expected to take up to 15 months. The last dose will be administered to the last subject approximately 6 months later (February 2008). The immunotherapy will be given orally to subjects who are likely to shed the organism in faeces at low levels for no longer than 7 days. Shedding constitutes the release, thus the release will end within 7 days of the last dose being administered to the last subject (February 2008). The clinical study will be completed with the final follow-up visit for the last subject 6 months after their last dose.

For details of the dosing schedule for individual subjects refer to Item 34.

36. The preparation of the site before the release.

The GMO will be administered to subjects at the clinical study sites which will be initiated according to GCP, including investigator training, prior to study start.

37. The size of the site.

Site 1 - CRC
The GMO will be administered at the Royal London Hospital in the Clinical Research Centre, Clinical Science Research Building, 2 Newark Street, London E1 2AT.

Site 2 – KCL
The GMO will be administered at King's College London School of Medicine, London SE5 9PJ.

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

At both clinical sites volunteers will receive the GMO by oral delivery, by drinking the GMO diluted in sodium bicarbonate solution. After an initial short observation period, the volunteers will be allowed to go home.

The GMOs may be shed from volunteers in faeces into the sewage system. Volunteers are restricted from leaving England for the first 14 days following dosing, beyond the period during which the GMO may be shed in faeces.
39. **The quantity of organisms to be released.**

   A maximum of 30 volunteers will receive up to $10^{10}$ CFU of the GMO on six occasions. The maximum total number of CFU administered will be $1.8 \times 10^{12}$ CFU. No replication or multiplication of the GMO is expected to occur prior to elimination of the organism from the body because of the attenuating mutations that have been introduced. While a large number of organisms consumed are likely to be shed as viable organisms in faeces, a substantial proportion of the organisms administered are unlikely to be shed as they will be taken up by local epithelial cells and the specialised antigen sampling areas of the gut known as Peyers patches, or will die during transit through the GI tract.

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.**

   Staff in direct contact with trial subjects will wear appropriate protective clothing (aprons and gloves). All staff will have Occupational Health clearance to work with hepatitis B infected patients.

42. **The post-release treatment of the site.**

   Following each administration of IMP, hard surfaces in the room used for administration will be disinfected. Disposable items will be incinerated or autoclaved.

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

   As a consequence of release, the GMO may be released into the sewage system. Potentially contaminated faeces released into the Public Mains Sewers will be treated according to Standard sewage treatment procedures.

   It is possible that some of shed organisms will enter environmental niches other than the sewage system, e.g. soil and water bodies. The GMO will not have a selective advantage in these environments and will not persist. Refer to Item 55 for discussion of the survivability of the GMO in these environments.

   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 evaluated in a clinical study to assess safety and immunogenicity in healthy volunteers. Release occurred as a consequence of administration of the GMO to the volunteers in this study, the GMO being shed in the faeces and so into the sewage system. The scale of this previous release was smaller than is intended for the proposed release. The previous release involved 30 subjects receiving up to 2 doses of $10^8$ CFU of the GMO over an approximate 5 month period. The proposed release is intended to involve up to 30 subjects receiving up to 6 doses of the GMO: one dose of $10^8$, one dose of $10^9$ and 4 doses of $10^{10}$ CFU (15 subjects will receive placebo). Dosing of all subjects is expected to occur over an approximate 21 month period.

Consent for deliberate release associated with the previous study was notified on 16 July 2003 (application reference number 02/R37/2, application made by Microscience Ltd, company name change to Emergent Europe Ltd, July 2005). Release occurred in accordance with the conditions applied to the consent for deliberate release.

Refer to Item 32 of this application for further information and results from the previous study.

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

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

**Site 1 - CRC**
The GMO will be administered at the Royal London Hospital in the Clinical Research Centre, Clinical Science Research Building, 2 Newark Street, London E1 2AT. The national grid appendice of this building is TQ346816. As a consequence of this release, the GMO may be released into the sewage system.

**Site 2 – KCL**
The GMO will be administered at King's College London School of Medicine, London SE5 9PJ. The national grid appendice of this site is TQ323760. As a consequence of this release, the GMO may be released into the sewage system.

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

**Site 1 - CRC**
The site is in central London.
**Site 2 - KCL**
The site is in south London, an urban area.

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

**Site 1 - CRC**
The site is approximately 1 mile from the River Thames.

**Site 2 - KCLC**
The site is within 1 mile of the River Thames.

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 organism is restricted in its host range to humans.

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

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

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

The natural habitat of the GMO, recipient and parent strains is humans. Humans will be the only recipients of the GMO. As a consequence of release, the GMO may be released into the public sewage treatment system and there is a possibility that the GMO could enter other environmental niches e.g. soil and water bodies.
53. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

*Site 1 - CRC*
No known developments

*Site 2 – KCL*
No known developments
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.

Wild type *S. typhi* only survive in the human host as there is no other animal vector and *S. typhi* can not persist in the environment. The method of dispersal for wild type *S. typhi* is dissemination and replication within the human host followed by shedding in faeces. Long-term shedding of the wild type occurs as a result of colonisation of the gall bladder which in turn is a result of wide dissemination of the organism during the bacteraemic phase of the infection. Replication within the gall bladder then leads to excretion of the organisms in the bile, and on into the faeces of the host. A proportion of individuals with a colonised gall bladder can become chronically infected carriers of *S. typhi*, resulting in persistent shedding.

The three modified traits present in the GMO are derived from the two attenuating deletion mutations in the *S. typhi* chromosome resulting in inactivation of genes A and B, and the insertion of the promoter-gene fusion in order to express the hepatitis B antigen. The deletion mutations result in the GMO being severely attenuated, unable to multiply within, or colonise the host. As the GMO can not multiply it can not give rise to systemic infections and colonise the gall bladder. As a result, shedding of the GMO only occurs transiently, and only a limited number of organisms are shed. Shedding occurs transiently as the GMO can not multiply in the digestive tract and so it is shed only as a result of the organisms passing directly through the GI tract following ingestion. Shedding therefore lasts only for the time it takes for GI transit to be completed. As the GMO can not replicate then the maximum number of organisms that can be shed will be limited and will be no greater than the number ingested. While the majority of the organisms consumed are likely to be shed as viable organisms in faeces, a substantial proportion are unlikely to be shed as they will be taken up by local epithelial cells and the specialised antigen sampling areas of the gut known as Peyers patches, or will die during transit through the GI tract. As the GMO can not colonise the gall bladder a chronic infection leading to the carrier state and persistent shedding can not occur.

Attenuation of both the GMO and the recipient has been demonstrated pre-clinically and in humans. Both strains have been assessed for safety and immunogenicity in clinical trials with healthy adult volunteers. In these studies both strains demonstrated a comparable safety profile, being well tolerated and safe in all subjects. The GMO was assessed in a Phase I study involving 30 healthy volunteers who received up to 2 doses of up to $1 \times 10^9$ CFU. The recipient strain has been assessed in three clinical trials involving a total of 89 healthy subjects who received up to $5 \times 10^9$ CFU. In all studies safety monitoring was a critical component and included monitoring the vaccinees closely for any signs or symptoms of infection related to the attenuated *S. typhi* strains.
Subjects were monitored for the presence of the organism in blood and urine, and stool samples were cultured regularly to establish the shedding profile in faeces and to determine whether there was persistent shedding (defined as shedding beyond day 7). This would be a sign that the organism had been able to establish a systemic infection leading to an infection of the gall bladder and consequent release of the organisms into the faeces. The data show that there were no bacteraemias and no persistent shedding; the organisms were not shed in faeces for longer than 7 days after each dose for any subject with either strain. To date the GMO has been administered to 30 healthy volunteers and the recipient strain has been administered to 89 volunteers. Details of the clinical studies and associated shedding profiles are given in Items 32 and 33(b), for the GMO and the recipient strain respectively.

A related Emergent Europe Ltd. S. typhi vaccine strain has also been assessed for safety and immunogenicity in a Phase I clinical study (performed under a CTX, Principal Investigator DJM Lewis; consent for deliberate release of a GMO reference 02/R37/1, consent notified on 14 March 2005 to Microscience Ltd. Microscience Ltd changed name to Emergent Europe Ltd in July 2005) and the data from this study provide support to the safety profile of the recipient strain and the GMO. This good safety profile is a reflection of the inability of these severely attenuated S. typhi strains to multiply within and colonise the human host.

The related vaccine strain is a candidate vaccine against traveller’s diarrhoea and is derived from the same recipient strain as the GMO. The vaccine is identical to the GMO except that it carries a different antigen within gene A, an ETEC (enteropathogenic Escherichia coli) antigen in place of the hepatitis B antigen. The traveller’s diarrhoea vaccine was administered to 36 healthy volunteers (see appendix 7 in the confidential annex); 12 subjects received 2 doses of $10^8$ CFU, 22 subjects received 2 doses of $10^9$ CFU and 2 subjects received a single dose of $10^9$ CFU. Subjects stayed in the Phase I unit (St George’s Vaccine Institute, London, UK) for 48 hours after each vaccination for close observation. Safety evaluations were focussed on the detection of the vaccine strain in blood, stool and urine. All subjects were required to take their temperature twice daily for 28 days following vaccination to detect any evidence of infection. Blood cultures were taken daily of the first 7 days and on Days 11, 14, 21 and 28 to detect any bacteraemias. Additional blood cultures would be taken if subjects showed signs or symptoms of an infection. No bacteraemias were detected for any subject. Stool samples were taken daily for the first 7 days and on Days 11, 14, 21 and 28 after each vaccination to determine the shedding profile. Overall there was no obvious difference between the number of subjects shedding, the frequency and duration of shedding, in relation to either the dose level or the number of doses administered.

Following administration of the first dose of the vaccine 22 of the 36 subjects shed on at least one day with the majority shedding over the first three days only. Sheding did not occur in any subject beyond Day 6:

- All 12 subjects in the low dose group shed the GMO; 10 subjects shed over the first 3 days only, 1 subject shed on Day 4 and 1 subject shed on Day 6.
Twenty of the 24 subjects in the high dose group shed the GMO; the majority of subjects did not shed beyond 3 days after dosing, 3 subjects shed over the first 4 days, 1 subject shed over the first 5 days, and 1 subject shed over the first 6 days.

Following administration of the second dose 28 of the 36 subjects shed on at least 1 day with the majority shedding over the first three days only.

- Eight of the 12 subjects in the low dose group shed the vaccine and shedding was within the first three days following dosing only.
- Twenty-one of the 22 subjects dosed with the high dose shed the vaccine; the majority of subjects did not shed beyond 3 days after dosing, 1 subject shed on Days 1, 2, 4 and 5 and 1 subject shed on Days 2, 3 and 14.

Therefore over the entire study (during which 70 doses of the vaccine were administered) positive stool cultures were not obtained for any subjects beyond 6 days after vaccination except in the case of one subject. For this subject the stool sample collected on day 14 after the second vaccination with $10^9$ CFU cultured positive for *S. typhi*. The subject remained well throughout the study with no evidence of infection with the vaccine. In line with the protocol the subject was treated with antibiotics and post-treatment blood and stool cultures cultured negative. This single, isolated incidence of a positive stool culture beyond day 7 after vaccination has not been observed for the recipient strain, 89 doses of which have been administered and follow-up by stool culture, nor for the GMO, of which 58 doses have been administered and followed-by by stool culture. This isolated positive stool culture post-day 7 is considered to be unusual and is an uncharacteristic finding for these severely attenuated *S. typhi* vaccine strains (see appendix 3 in the confidential annex).

There is limited published data available on the shedding profile of the only licensed live attenuated typhoid vaccine, *S. typhi* Ty21a (Vivotif Berna™, Berna). This vaccine is derived from the same parent strain as the recipient strain, *S. typhi* Ty2, and has proved to be a successful vaccine for the prevention of typhoid fever. However, it was attenuated using rounds of chemical mutagenesis rather than rational attenuation through targeted genetic mutations and the exact mechanism of attenuation is unknown. The two published papers that are available report no detection of the vaccine strain from the stools of subjects, however these papers do not provide any information on detection or culture methods used. The lack of shedding may reflect the overly attenuated nature of the strain, owing to the crude method of attenuation; this is reflected in the requirement for multiple doses (3-4) in order to achieve a protective immune response. The limited published data available on the shedding of Ty21a are summarized below.

As part of a field study of Ty21a conducted in Egypt in 1978-1979, a pilot study was conducted in 884 children. 413 received the vaccine and 471 received placebo. Vaccine recipients received three oral doses, every other day, consisting of $1-8 \times 10^9$ CFU. From the 884 students, it was possible to collect 510 stool samples 7 days after the first dose (ie 2 days after the third dose), and 475 stool samples after another 2 weeks. The vaccine strain was not isolated from any of the stools. It is not stated how many of these subjects were in the vaccine group and how many in placebo. No culture methods are given (Wahdan, MH et al, 1980).
In studies carried out in healthy US college students, 141 adults received vaccine in either of two ways, in gelatin capsules or in enteric coated capsules. 36 subjects received one dose, 30 received 2 doses and 16 received 3 doses of enteric coated capsules, while 44 subjects received 1 dose and 15 received 2 doses of gelatin capsules. Ty21a was not very immunogenic in these individuals (23% seroconversion) and the vaccine strain was not recovered from the stool cultures of any of the volunteers. However, it is not stated when the stool samples were taken, and the culture methods used are not given (Black, R et al, 1983).

As described above, during the proposed clinical study with the GMO limited numbers of the organism could be shed in subject’s faeces and it is expected that this will be for no longer than 7 days after administration; reflecting the transit time of the organism directly through the GI tract. The GMO could therefore be released into the sewage system and available data indicate that the GMO will not survive or persist in the sewage system. As with wild type S. typhi entering the sewage system in the faeces of patients with typhoid fever, it is anticipated that the GMO will be effectively contained and inactivated by normal sewage treatment processes. Additionally, Emergent Europe has performed a study to ascertain survival of the GMO in raw, untreated sewage. Results of this study showed a steep drop in viable cell numbers of the GMO between Day 0 (when the GMO was added to the sewage) and Day 1. During this time cell numbers dropped from approximately 87% of the total population of organisms to less than 1%. By Day 2 the GMO could no longer be detected amongst the native sewage organisms, and was not recovered in any further samples taken up to and including Day 25 post-inoculation. There was therefore no evidence of survival of the GMO in raw sewage beyond Day 1 confirming that this strain does not persist or have a survival advantage in this environment (refer to Item 55 for detail of this study).

It is acknowledged that GMOs entering the sewage system have the potential to come into contact with other environmental niches. In addition, it could be possible that some faecal samples containing the GMO may be disposed of by means that do not involve a mains sewer and from here the GMO may enter other environmental niches. Emergent Europe has shown that the recipient strain and the GMO do not survive in soil and water and available data indicate that the strain will be unable to survive or persist in sea water and river water. Wild type S. typhi is unable to persist in the environment outside the human host and the highly weakened GMO will not have a survival advantage over wild type; due the additional nutritional requirements of the GMO compared to wild type, as a result of the gene A mutation, the GMO may indeed be expected to have a survival disadvantage compared to wild type. Refer to Item 55 for a more detailed discussion of the survivability of the GMO in the environment.

In summary, the GMO is severely attenuated, unable to survive within and colonise humans, the only host of wild type S. typhi, and unable persist in the environment outside the host. In the proposed clinical study dispersal of the GMO will occur via shedding in faeces of the subjects. The majority of shedding will occur within the first 3 days following administration and is extremely unlikely to occur beyond 7 days after dosing.
However, subjects will be closely monitored to assess safety of the GMO and if at any time the Investigator considers a subject has a clinical *S. typhi* infection (which could theoretically lead to shedding of the GMO in stools), the subject will be treated with antibiotics and followed-up until the infection is clear. In addition, stool samples are scheduled to be cultured 6 months following the final dose (to ensure the subject has not become a chronic carrier of the GMO), and when clinically indicated, at the discretion of the Investigator. If at any time a stool sample obtained beyond 7 days after administration of the GMO cultures positive, subjects will be treated with antibiotics and followed-up until the infection is clear. Should the GMO enter the sewage system or other environmental niches such as soil or water this strain will not persist; wild type *S. typhi* does not persist in the environment and the GMO, a weakened form of *S. typhi* will not have a survival advantage over wild type.

In addition to the above information, appropriate contained use risk assessments have been performed on the recipient strain as well as the GMO and in both cases the organisms have been classified as Class I (containment level 1). Furthermore, the risk assessment for the recipient strain, which is derived from wild-type *S. typhi*, has been submitted to the UK Health and Safety Executive (HSE) and there were no objections to this risk assessment. In accordance with the HSE guidelines activities with Class I organisms are activities which pose “no or negligible risk”.

**References**


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

Following administration to humans the vaccine strain will be released into the sewage system through the faeces of subjects. The GMO is likely to be shed in faeces in small numbers for a very limited time (not beyond 7 days) and it is expected that the majority of the GMO organisms will be contained within the sewage system and inactivated by the normal sewage treatment processes as is the case for wild type *S. typhi*. The stools from any patient suffering from typhoid fever disease are discharged into the sewage system and wild type *S. typhi* within them is effectively contained and inactivated by normal sewage treatment processes as is demonstrated by the fact that the UK sewage system has significantly contributed to the eradication of enteric diseases such as typhoid and cholera from the UK population. However, despite this it is essential to consider how the sewage environment will affect survival of the GMO strain itself and so Emergent Europe has performed a study to characterise persistence of the GMO in raw sewage as described below.
A raw sewage sample containing $3 \times 10^6$ colony forming units per millilitre (CFU/mL) of naturally occurring raw sewage organisms was spiked with the GMO to give a ratio of GMO to raw sewage organisms of approximately 1:1. The spiked material was held at ambient temperature (~20°C) without agitation in the dark, and aliquots removed at Days 0, 1, 2, 3, 5, 7 and 25. At each timepoint approximately 100 colonies were selected at random and the number of GMO colonies within that sample was determined by screening for dependency for aromatic compounds (the nutritional requirement resulting from the gene A mutation). The number of isolates identified as the GMO at each timepoint is given in Table 1.

Table 1: Summary of Results for the Persistence of the GMO in Raw Sewage

<table>
<thead>
<tr>
<th>Timepoint (Days)</th>
<th>Mean Estimated Total Viable Count (CFU/mL)</th>
<th>Total Number of Isolates Analysed</th>
<th>Number of Isolates Identified as the GMO</th>
<th>Percentage of isolates identified as the GMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>$1.85 \times 10^6$</td>
<td>124</td>
<td>108</td>
<td>87</td>
</tr>
<tr>
<td>1</td>
<td>$8.73 \times 10^5$</td>
<td>139</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>$4.27 \times 10^5$</td>
<td>124</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>$4.58 \times 10^5$</td>
<td>138</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>$2.69 \times 10^5$</td>
<td>142</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>$1.76 \times 10^5$</td>
<td>123</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>$1.19 \times 10^4$</td>
<td>101</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

On Day 0 the number of isolates identified as the GMO represented approximately 87% of the total number of isolates analysed. At the Day 1 timepoint, the number of isolates identified as the GMO had fallen to <1% of the isolates picked. This represents a 99.6% loss of viable cells in ~24 hours. At the Day 2 timepoint no GMO colonies were detected, representing an additional loss of >0.4% of the GMO subpopulation between the Day 1 and Day 2 timepoints. From the Day 2 timepoint onwards, no further evidence of the GMO was detected in the spiked sewage.

In summary, over the course of the study, microbiological analysis of the GMO and raw sewage mixtures revealed that the GMO population fell by 99.6% after storage for one day. By Day 2 the GMO sub-population fell further to less than the limit of detection (approximately 1%) and was not detected at any timepoint beyond Day 2. It is therefore reasonable to conclude that the GMO does not persist in the raw sewage environment for more than two days and it does not have a competitive advantage in sewage over the naturally occurring organisms. These data are in-line with the data submitted in the previously approved application 02/R37/2 which demonstrated that the 95.4% of the GMO organisms were killed after 9 days. The data in application 02/R37/2 were obtained in a provisional study which was later repeated to include more frequent sampling over a longer period of time.
Emergent Europe acknowledges that GMOs entering the sewage system have the potential to come into contact with other environmental niches. In addition it could be possible that some faecal samples containing the GMO may be disposed of via facilities that do not involve a mains sewage system and from here the GMO may enter other environmental niches. In respect to this the GMO will not have a survival advantage over wild type \textit{S. typhi}. Wild type \textit{S. typhi} does not have any reservoirs other than the human body and does not persist in the environment. Nevertheless, Emergent Europe has carefully considered any potential risk should the GMO come into contact with environmental niches such as soil or water bodies.

Emergent Europe has undertaken studies to examine the survival of both the recipient strain and the GMO in soil. Using sterile topsoil the recipient strain was shown to persist for only 8 days. Studies looking at persistence of the GMO were performed using non-sterile topsoil to more accurately reflect the natural environment. These studies showed that the GMO did not persist for longer than 12 days. See \textit{appendix 8 in the confidential annex} and \textit{appendix 41 in the confidential annex of previously approved application 02/R37/2}.

With regard to potential contact with water bodies, the Environment Agency has confirmed that any sewage that is discharged into coastal areas is UV irradiated, prior to discharge, in order to meet EU requirements. In the unlikely event that any GMOs were still surviving at this point following sewage treatment, UV irradiation would kill them. The worst-case scenario is that some GMOs will reach seawater however they would not be expected to persist. A study carried out by Wait and Sobsey (2001) compared the survival of enteric bacteria and viruses in seawater and demonstrated in an \textit{in situ} field study that there was no significant difference between the inactivation of \textit{E. coli} and \textit{S. typhi} and that \textit{S. typhi} was inactivated between 1 and 4 days indicating that it does not persist in this environmental niche.

Published data are also available on survival of \textit{S. typhi} in soil and river water. Acambis Research Ltd has published data on an attenuated \textit{Salmonella typhi} vaccine strain ACAM948-CVD (Part B Application to DEFRA – see DEFRA website). This vaccine strain contains a deletion in the A gene. It also contains a mutation in the \textit{htrA} gene. This mutation, like the gene B mutation in the Emergent Europe product, affects the ability of \textit{Salmonella} to survive in macrophages. Acambis Research Ltd presented data on the survival in soil and river water of the wild-type \textit{S. typhi} Ty2 and of the parent strain of ACAM948-CVD, which is phenotypically equivalent to an \textit{S. typhi} strain containing a deletion in gene A only. These data show that for both strains in soil 99% of organisms were dead after 14 days and none were recoverable after 3 weeks. In river water neither strain was isolable after 14 days. Further data presented show that ACAM948-CVD survives in river water for a maximum of 5 days. Given the similarities between ACAM948-CVD and the Emergent Europe recipient strain it can be expected that survival of the Emergent Europe recipient strain in river water would be similar. Previous studies have shown that the Emergent Europe recipient strain and the Emergent Europe GMO act in a comparable manner, therefore it is reasonable to expect that survival of the GMO in river water would not exceed that of the GMO recipient strain, wild type \textit{S. typhi} Ty2, or ACAM948-CVD.
With regard to standard community drinking water supplies (the public mains water system), again available data indicate that the GMO will not survive in this environment. Standard community drinking water is chlorinated and Emergent Europe have generated data showing that the recipient strain survived in tap water for less than 2 days and survived in water freshly chlorinated with 0.2mg/mL chlorine, a chlorine concentration below that recommended for drinking water supplies, for less than 5 minutes (see appendix 9 in this confidential annex and appendix 21 in the confidential annex of previously approved application 02/R37/2).

To conclude:
- The GMO will enter the sewage system. Stools from any patient suffering from wild type typhoid infection are discharged into the sewage system and the UK sewage system effectively contains and inactivates these organisms. Experimental data has shown that the GMO does not persist or have a survival advantage in sewage.
- It is possible that the GMO could come into contact with aspects of the environment other than the sewage system e.g. soil and water bodies. Wild type \textit{S. typhi} is known not to persist in the environment and all available data indicates that the highly attenuated Emergent Europe GMO will also not persist in the environment.
  - Experimental data has shown that the GMO was not detectable in soil samples after 12 days.
  - Data on a similarly attenuated organism (ACAM948-CVD) has demonstrated that the organism was unable to survive more than 5 days in river water; it is expected that the Emergent Europe GMO would have a similar survival profile.
  - Wait and Sobsey (2001) have published data to show that \textit{S. typhi} cannot persist in sea water.
  - Experimental data has shown that the recipient strain was killed in (chlorinated) tap water and the GMO would be expected to have a similar survival profile.
- The GMO, which is a highly weakened form of \textit{S. typhi}, is not expected to persist in the environment.

References
Wait DA, Sobsey MD. \textit{Comparative survival of enteric viruses and bacteria in Atlantic Ocean seawater}. \textit{Water Science and Technology} 2001; 43(12): 139-142

56. \textbf{The sensitivity to specific agents.}

This information is provided in the equivalent section of the previous application 02/R37/2.

\textbf{Interactions with the environment}

57. \textbf{The predicted habitat of the organism.}

This information is provided in the equivalent section of the previous application 02/R37/2.
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.

This information is provided in the equivalent section of the previous application 02/R37/2.

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

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

This information is provided in the equivalent section of the previous application 02/R37/2.

(b) from indigenous organisms to the genetically modified organisms.

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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.

This information is provided in the equivalent section of the previous application 02/R37/2.

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

Humans are the only host for wild type S. typhi, there are no animal vectors and it is unable to persist in the environment. Wild type S. typhi is dispersed via faecal-oral transmission, the organisms being shed in very large numbers in the faeces of the diseased individual. The GMO harbours two distantly located mutations that attenuate the strain preventing replication and therefore dispersal of the GMO. The attenuated strain is non-pathogenic in humans and faecal shedding occurs in extremely low numbers and for a very limited duration (less than 7 days). 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 young and elderly (refer to Item 68 for more details of the exclusion criteria).
Volunteers will also be instructed to maintain strict personal hygiene and proper hand washing will be stressed to minimise the risk of faecal-oral transmission.

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

As a consequence of release, the GMO may be released into the sewage system. There is also a possibility that the GMO could enter other environmental niches such as soil and water bodies.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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 interaction.

A consideration for the GMO is faecal-oral transmission from the volunteers to non-target hosts. 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. Women who are breastfeeding and individuals who work as commercial food handlers are excluded. Individuals who are health care workers with direct contact with high-risk patients, child care workers or other individuals who have regular contact with children less than 2 years of age, and individuals with household contacts with immuno-compromised individuals, pregnant women or the young and elderly will all be excluded from the trial.
Volunteers will also be instructed to maintain strict personal hygiene and proper hand washing will be stressed to minimise the risk of faecal-oral transmission (see appendix 6 in the confidential annex).

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

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.

71. The known or predicted involvement of the organisms in biogeochemical processes.

This information is provided in the equivalent section of the previous application 02/R37/2.

72. Any other potential interactions of the organisms with the environment.

This information is provided in the equivalent section of the previous application 02/R37/2.
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.

Extensive assay development work has been performed to ensure the GMO and the related attenuated *S. typhi* strains (based on strains containing the gene A and gene B deletion mutations) can be detected through *in vitro* culture methods. Once the organism has been cultured specific PCR techniques can then be applied to identify the strain. This ensures it is possible to detect and monitor the presence of these strains in the stools of the volunteers who participate in the clinical studies, and in environmental niches such as sewage and water.

Additionally, 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.

Detection of the recipient strain the GMO and a related strain in stools

The results of development work show that the stool culture assay is sensitive and will effectively detect low levels of the recipient strain, the GMO and the related traveller’s diarrhoea *S. typhi* vaccine strain in stool samples. The stool culture assay is comprised of two parts: the direct plating step which involves plating the stool sample directly onto selective agar medium, and the enrichment step which involves culturing the sample overnight in selective liquid medium prior to plating onto the selective agar medium.

To date shedding data has been generated in 3 clinical studies with the recipient strain, 1 study with the GMO and 1 study with the related traveller’s diarrhoea vaccine strain. For the first clinical study with the recipient strain both the direct plating agar medium and the liquid enrichment medium were used without aromatic supplements. For all further studies with the recipient strain, the GMO and the related traveller’s diarrhoea vaccine strain the media were supplemented with aromatic compounds for use. The aromatic supplement was added as the *S. typhi* strains have a nutritional requirement for aromatic compounds which may not be met by the non-supplemented media. Therefore, although the recipient strain was shown to grow well on these media without aromatic compounds, the aromatic supplements were added to maximize the chance of recovering these *S. typhi* strains.

The initial clinical study with the recipient strain (Hindle *et al*. 2002, see Item 33(b)) used standard microbiological techniques to detect the strain. Briefly, this involved inoculating faecal samples directly onto desoxycholate citrate agar (DCA) and xylose lysine desoxycholate (XLD) agar (direct plating method) and enriching in selenite-F enrichment broth prior to plating onto DCA plates (enrichment method).
Aromatic supplements were not used in the DCA or XLD media used in this study however preliminary studies performed prior to the start of the study had shown that it was possible to detect the strain on these media without aromatic supplements. This study involved only a small number of subjects and now contributes only a small proportion of the data on shedding of the recipient strain. The shedding profiles observed in this study were comparable to those observed in later studies.

Prior to the second clinical study (Phase I) with the recipient strain (Kirkpatrick et al. 2006, see Item 33(b)), the sensitivity of the assay was determined at the two Investigator sites involved, by spiking stool samples with known amounts of the organism and looking for recovery using the culture method to be used in the study. Human stool samples were spiked with the recipient strain at concentrations from $1 \times 10^4$ CFU/mL to 5 CFU/mL. Spiked samples were then plated directly onto DCAaro agar plates or enriched in selenite-Faro broth prior to plating onto DCAaro agar plates. *S. typhi* like colonies were isolated from the DCAaro plates and used to inoculate Triple Sugar Iron (TSI) agar slants and BHIaro plates. TSI agar allows *S. typhi* to be differentiated from other bacteria that give phenotypically similar colonies on DCAaro plates. The identity of the recovered cells was confirmed using slide agglutination tests (test material taken from the BHIaro plate) to detect O-9, Vi and d-H antigens on the cell surface.

Results from one centre demonstrated that the recipient strain could be detected at levels of $5 \times 10^2$ CFU/mL of stool sample by directly plating onto DCAaro agar and can be detected at levels of 5 CFU/mL when enriched in Selenite Faro broth and then plated on DCAaro agar. Results from the other centre indicated that the recipient strain could be detected at levels of $2 \times 10^3$ CFU/mL of stool sample by direct plating and can be detected at levels of $5 \times 10^1$ CFU/mL by the enrichment method. These results indicate that the assay is sensitive and will effectively detect low levels of the recipient strain in stool samples. The same stool culture assay was used in the second Phase I study performed with the recipient strain (Kirkpatrick et al. 2005, see Item 33(b)).

A similar sensitivity assessment was made for the GMO prior to the start of the Phase I study with this strain (refer to Item 32 for details of this study). The stool culture method used for the GMO involves media supplemented with aromatic compounds and is the same as that used for the recipient strain in the two US Phase I studies, except that colonies are not screened using TSI slants prior to agglutination testing. In the sensitivity study, performed at the clinical unit where the Phase I trial was run, the GMO was spiked into human stool at concentrations of 3300 CFU to 33 CFU per stool sample for the direct plating method (DCAaro agar) and 1100 CFU to 11 CFU per stool sample for the enrichment method (selenite-Faro broth plus DCAaro agar). The GMO was recovered at the lowest level tested for both the direct plating and enrichment steps, demonstrating that the assay sensitivity is less than or equal to 33 CFU per stool sample for the direct plating step and less than or equal to 11 CFU per stool sample for the enrichment step. This gives an overall assay sensitivity of less than or equal to 11 CFU per stool sample for recovery of the GMO.
The assay used for detection of the related, traveller’s diarrhoea vaccine strain is the same as that used for detection of the GMO. When the assay sensitivity was assessed for recovery of the related strain, lower cell concentrations were used than had previously been tested when assessing sensitivity for recovery of the recipient and the GMO: concentrations in the range of 41 CFU per stool sample to 0.2 CFU per stool sample were applied to each method.

The sensitivity of the direct plating method (DCAaro) was found to be 41 CFU per stool sample and the sensitivity of the enrichment method (selenite-F broth plus DCAaro) was 0.4 CFU per stool sample, giving an overall assay sensitivity of 0.4 CFU per stool sample for recovery of the related strain.

Prior to initiation of the proposed Phase II trial with the GMO the assessment of assay sensitivity will be repeated for recovery of the GMO; lower cell concentrations will be used so that a more precise estimate of sensitivity can be obtained. As in the Phase I study the GMO will be plated onto DCAaro for detection in the direct plating method, and will be enriched in selenite-Faro broth prior to plating onto DCAaro for detection in the enrichment method.

The results of the development work described above demonstrate that using these stool culture methods all three attenuated S. typhi strains can be readily detected when present at low levels in faecal stool samples. The same methods are suitable for detection of the organisms from sewage samples.

Using these sensitive assay methods the shedding profile of each of the three strains was assessed in Phase I studies, and it was established that shedding of these strains in faeces is limited in duration, and is extremely unlikely to occur beyond 7 days after administration (refer to Item 54).

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.**

This information is provided in the equivalent section of the previous application 02/R37/2.

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

This information is provided in the equivalent section of the previous application 02/R37/2.
76. **Duration and frequency of the monitoring.**

During the clinical trial subjects will receive up to 6 doses of the GMO (or placebo) at 28-day intervals over a 6-month period. The clinical trial procedures ensure close clinical, haematological, biochemical, physiological monitoring of immunised volunteers for any adverse effect. After each dose it is possible that the GMO will be transiently shed in faeces. Clinical data obtained for the GMO, the recipient strain and a related strain show that shedding will occur for no more than 7 days following each dose (refer to **Items 32, 33(b) and 54** for details). Shedding is transient as the GMO is severely attenuated and unable to multiply within, or colonise the human host.

The shedding profile obtained following administration of the GMO contrasts greatly with the pattern resulting from infection with wild type *S. typhi*, and this contrast is due to the severe attenuation of the GMO. In a proportion of infected individuals, wild type *S. typhi* is able to colonise the gall bladder where the cells replicate and then enter the bile which carries the organism to the GI tract from which they are shed in faeces. Long term shedding with wild type *S. typhi* is therefore a result of gall bladder infection which in turn occurs as a result of the organisms being widely disseminated during the bacteraemic phase of an infection which is associated with fever and other clinical symptoms. As the GMO is severely attenuated and does not give rise to systemic infections and bacteraemias, it can not colonise the gall bladder. Shedding of the GMO in faeces therefore only occurs as a result of the organisms passing directly through the GI tract following ingestion, and lasts only for the time it takes GI transit to be completed.

During the clinical study subjects will be carefully monitored for signs and symptoms of a clinical infection with *S. typhi*, as this could theoretically lead to shedding of the GMO in stools (release of the GMO). If at any time a subject is considered to be clinically infected, blood, urine and stool will be cultured to monitor for the GMO, and the subject will be treated with antibiotics. Following the course of therapy further monitoring will be performed; subjects will have further stool, blood and urine samples cultured and for the infection to be considered clear all samples must be negative for *S. typhi* on two consecutive days.

Safety assessments are therefore a critical component of the planned study, vital for monitoring the subjects for signs and symptoms of a clinical infection with *S. typhi*. After each dose subjects will visit the clinic for regular assessments; they will also be instructed to contact the study site if they become unwell between scheduled visits. The Investigator may then arrange for the subject to attend clinic for an additional safety assessment, and blood, stool or urine samples will be taken for culture if clinically indicated. In this way the subjects are closely monitored, and appropriately assessed and treated should they develop signs or symptoms of a clinical infection with *S. typhi*. 

**Safety assessments**

The scheduled visits are on Days 3, 7, 14 and 28 after the first dose; Days 7, 14, and 28 after the second dose and Days 14 and 28 after subsequent doses (a maximum of 6 doses will be administered). Following the final dose there are additional visits at 2, 4 and 6 months post-dosing. At each scheduled visit safety will be assessed by vital signs (pulse, blood pressure and body temperature), serum biochemistry and haematology tests, urine dipstick testing and a review of adverse events. All adverse events must be monitored until there is either a return to normal or until the condition becomes stable or is resolved. Should the urine dipstick give a positive result for leucocyte esterase and nitrites, a fresh sample of urine will be cultured to establish whether an *S. typhi* infection has developed.

At the final study visit (6 months after the final dose) the above safety assessments will be made and the subjects will have a standard physical examination. Stool samples will also be cultured to confirm subjects have not become chronic carriers of the GMO.

At unscheduled visits blood, stool or urine samples may be taken for culture if clinically indicated (as described above) and in addition blood may be drawn to repeat laboratory tests carried out at routine visits.

**Antibiotic treatment**

If a subject has a stool culture that is positive for *S. typhi* more than 7 days after receiving their most recent dose of the GMO, they will be treated with antibiotics (as described below). Stools will only be cultured for the following reasons: at the discretion of the Investigator following examination of a subject during a scheduled or an unscheduled visit; as part of the safety assessment 6 months after the final dose.

If, at any time, a subject is considered to have a clinically significant infection with the GMO he/she will be treated with antibiotics and followed up until the infection is clear.

The protocol states that a subject is considered to be infected if:
- A single blood culture is positive for *S. typhi* and the patient displays clinical signs and/or symptoms of infection
- The Investigator considers the patient to be infected

Following the course of therapy the subjects will have blood, stool and urine samples cultured. For the infection to be considered clear all blood, stool and urine samples must be negative for *S. typhi* on two consecutive days.

Antibiotic treatment will be as follows. For subjects with a bacteraemia, ciprofloxacin 500 mg will be given twice daily for 10 to 14 days. Should this regimen not be effective or the patient is sensitive to ciprofloxacin the subject will be treated with the combination of trimethoprim 160 mg and sulfamethoxazole 800 mg twice daily for 7 to 14 days. The GMO has been shown to be sensitive to ciprofloxacin and the trimethoprim/sulfamethoxazole combination. The minimum inhibitory concentration (MIC) for ciprofloxacin is 0.012 µg/mL. The MIC for trimethoprim/sulfamethoxazole is 0.032 µg/mL
Monitoring will therefore be for the duration of the study and will be continuous as subjects will be requested to call study staff any time they are concerned about their health.

**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.

Experiments have been carried out that indicate that 70% isopropanol is an efficient sterilising agent, killing the recipient strain and the GMO. Aerosol spray delivery of 70% isopropanol effectively sterilises contaminated bench top surfaces, fabric or paper. For all surfaces, including inaccessible surfaces, as in the tubing and eppendorfs, or potentially pitted surfaces, as with the stainless steel, immersion into 70% isopropanol for a minimum of 10 seconds kills the organisms completely. Also the organisms persisted on formica bench surfaces for only short periods of time and were not detected after 15 days. *(See appendices 39 and 40 in the confidential annex of the previous application 02/R37/2).*

The GMO (in a liquid formulation) will be administered in a room with separate hand-washing facilities. All clinical waste, including tissues, disposable clothing and other personal protective equipment and all items that may have been in contact with the GMO, will be contained within closed bins until disposal by autoclaving or incineration. Only those staff registered by the local Advisory Committee of Genetic Manipulation are permitted to enter the clinical room during the study days.

Following dosing, patients will stay in the clinical room for 1 hour. The use of personal items such as mobile phones, magazines, books etc. will not be permitted during this period. This will reduce the potential for secondary spread of the GMO. The waste from the sanitary facilities directly enters the public sewers that are capable of containing and inactivating the GMO.

After the patients leave the clinical site, faeces will also enter the public sewers. Patients will be educated in hand-washing and other methods to avoid secondary transmission of the GMO using the Food Standard Agency guidance on hand washing for food preparation.

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

Only those staff registered by the local Advisory Committee of Genetic Manipulation will be permitted to enter the clinical room during the study days. These designated staff will prevent access to the room by unauthorised personnel.
79. Methods and procedures to prevent other organisms from entering the site.

All staff engaged in the study will receive appropriate training regarding their own safety (and that of other persons who might be affected by their actions) and regarding precautions to be taken to ensure: no contamination will result from the activities associated with the dose administration; nothing will occur that could interfere with the outcome of the study; all materials are disposed of in a suitable manner.

Staff are encouraged to participate in health and safety issues via the Safety representatives or by their participation in the various committees.

Waste treatment

80. Type of waste generated.

- Laboratory waste (plastic ware, liquid reagents, microbial cultures).
- Clinical waste (faecal, urine, blood samples).
- Miscellaneous waste (disposable clothing, tissues).

81. Expected amount of waste.

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

82. Description of treatment envisaged.

- Laboratory waste: Laboratory waste will be placed in a container and autoclaved prior to being incinerated.
- Clinical waste: Blood, faecal and urine samples collected into lidded, plastic containers for analysis will be autoclaved and then incinerated.
- Miscellaneous waste: All items that have the potential for contamination, such as disposable clothing, tissues etc will be placed in sealed bags/containers prior to autoclaving and incineration.

Emergency response plans

83. Methods and procedures for controlling the organisms in case of unexpected spread.

Transmission of the GMO would be solely human to human with no animal, plant or insect vector. All control measures will be in place to identify persons who have become infected with the GMO, are carriers of the GMO or are susceptible to infection with the GMO.
If any of the volunteers vomit following administration of the GMO this will be treated as a biological hazard and suitable protective clothing and disinfectant will be used to inactivate the hazard with all items being disposed of into sealed containers for autoclaving and incineration. However since the GMO is severely attenuated it will not survive outside the human host.

The GMO is sensitive to ciprofloxacin, an antibiotic that is licensed for human use in the event of infection with Salmonella. This antibiotic is effective in the treatment of acute infection and eliminating chronic carriage. In the case of children, alternative effective antibiotics (e.g. ampicillin) are also available.

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

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

Contaminated areas maybe decontaminated by the use of standard disinfectants. The efficacy of the disinfectant can be tested by swabbing the disinfected area and inoculation into appropriate media.

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

Contaminated areas may be decontaminated by the use of standard disinfectants (see appendices 39 and 40 in the confidential annex of previously approved application 02/R37/2; e.g. hypochlorite solution, Virkon, Actichlor).

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

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

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

The exclusion criteria in the protocol for study MS04.03 state that a patient will be excluded if they “Plan to leave England (even for a day trip) within 14 days following each dosing day”. Before participating in the study, each subject will sign a consent form agreeing to remain within England for a period of 14 days following each dosing day during the study. As a precautionary measure, if required, any subject who needed to leave England in an emergency within the period of 14 days following each dosing day could be treated with prophylactic antibiotics.
Subjects will be monitored for signs/symptoms of infection with the vaccine strain throughout the study; should such an infection be observed, the subject concerned would be assessed and treated with antibiotics as appropriate for the infection.

87. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

If any of the volunteers vomit following vaccination this will be treated as a biological hazard and a suitable disinfectant will be used to inactivate the hazard. All decontamination and disposal procedures will be performed wearing suitable protective equipment in accordance with clinical study site safety codes. However, since the GMO is severely attenuated it will not survive outside of the human host.

The clinical trial procedures ensure close clinical, haematological, biochemical physiological and microbiological monitoring of immunised patients for any adverse events. If at any time the Investigator believes a volunteer to be infected with the GMO (such an infection may result in persistent faecal shedding (beyond 7 days)), he will administer antibiotics and blood, stool and urine samples will be cultured. Following the course of antibiotic therapy further blood, stool and urine samples will be cultured and for the infection to be considered clear all samples must be negative for *S. typhi* on two consecutive days.

If required, facilities will be available at the site to admit to hospital any subject manifesting an undesirable effect. This will be done under quarantine if necessary.

In addition to the above information, appropriate contained use risk assessments have been performed on the recipient strain as well as the GMO and in both cases the organisms have been classified as Class I (containment level 1). Furthermore, the risk assessment for the recipient strain, which is derived from wild-type *S. typhi*, has been submitted to the UK Health and Safety Executive (HSE) and there were no objections to this risk assessment. In accordance with the HSE guidelines activities with Class I organisms are activities which pose “no or negligible risk”.
Part VI

Information on Methodology

Information on standard microbiological culture medium is provided in item 7.

Stool culture methods are described in Item 73 of this application.

Information on other methods is provided in the equivalent section of the previous application 02/R37/2.