SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

## A. GENERAL INFORMATION

### 1. Details of notification

| | | |
|---|---|---|
| (a) | Member State of notification: | UK |
| (b) | Notification number: | B/GB/02/37/02 |
| (c) | Date of acknowledgement of notification | |
| (d) | Title of the project: | An open-label study to determine the safety and immunogenicity of two dose levels ($10^8$ or $10^9$ CFU) of a candidate oral immunotherapy (Hep B Candidate 1) against hepatitis B, given on two occasions, 56 days apart to healthy subjects |
| (e) | Proposed period of release: | April 2003 – June 2004 |

### 2. Notifier

| |
|---|
| Name of institution or company: Microscience Ltd |

3. GMO characterization

| | | |
|---|---|---|
| (a) Indicate whether the GMO is a: | Viroid | ☐ |
| | RNA virus | ☐ |
| | DNA virus | ☐ |
| | bacterium | ☑ |
| | fungus | ☐ |
| | animal | ☐ |
| | - mammals | ☐ |
| | - insect | ☐ |
| | - fish | ☐ |
| | - other animal | ☐ please specify phylum, class |
| | other, please specify (kingdom, phylum and class) | |
| (b) Identity of the GMO (genus and species)<br>Enterobacteriaceae: Salmonella enterica | | |
| (c) Genetic stability – according to Annex IIIa, II, A(10)<br>PCR evidence suggests that the gene A and gene B deletion mutations and the promoter- gene fusion inserted into the chromosome of the GMO are stable over at least 10 days of passaging. Not only are the genetic modifications of the GMO stable over this period, but the phenotypic characteristics exhibited by possessing a deleted gene A mutation also remain the same (ie auxotrophic requirements). Genetic stability during manufacture of the GMO is demonstrated for each batch. | | |

4. Is the same GMO release planned elsewhere in the Community [in conformity with Article 6 (1)], by the same notifier?

| Yes ☐ | No ☑ |
|---|---|
| If yes, insert the country code(s): | |

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

| Yes ☐ | No ☑ |
|---|---|
| If yes:<br>- Member State of notification<br>- Notification number | |

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

| Yes ☐ | No ☑ |
|---|---|
| If yes:<br>- Member State of notification<br>- Notification number | |

7. Summary of the potential environmental impact of the release of the GMOs

The GMO is a severely attenuated strain of the human-specific pathogen *Salmonella typhi* expressing a Hepatitis B virus antigen and is intended for use as an immunotherapy in chronic carriers. Hep B Candidate 1 will be given orally to volunteers who are likely to shed the organism in stools at low levels for no longer than 7 days. Shedding will constitute the release of the organism and potentially, it could be released into the Greater London Sewage system.

The potential for genetic exchange with any other organisms in the environment is extremely low, given that the GMO does not contain any plasmids (or antibiotic resistance markers) and does not persist in the environment for very long.

The advantage of using attenuated *Salmonella typhi* as the vector is that there are no animal reservoirs and it does not persist in the environment. Wild type *S. typhi* does not persist or replicate in chlorinated water, in fact chlorination of water has had a major impact in eradicating typhoid fever as an endemic disease in countries such as the US. It has been demonstrated that the recipient strain and the GMO do not replicate or persist in chlorinated tap water.

The information presented in this application demonstrates the safety of the GMO, in terms of its inability to cause harm and to persist in the environment. Clearly it is the key objective of the clinical trial to assess the safety of the GMO in human volunteers. The risk assessment shows a low hazard associated with administering the GMO to human volunteers and the risk to the environment is considered to be effectively zero.

B. INFORMATION RELATING TO THE RECIPIENT OR PARENTAL ORGANISMS FROM WHICH THE GMO IS DERIVED

1. Recipient or parental organism characterization:

(a) Indicate whether the recipient or parental organism is a:

| | |
|---|---|
| Viroid | ☐ |
| RNA virus | ☐ |
| DNA virus | ☐ |
| bacterium | ☑ |
| fungus | ☐ |
| animal | ☐ |
| - mammals | ☐ |
| - insect | ☐ |
| - fish | ☐ |
| -other animal | ☐ (please specify phylum, class) |

other, please specify

2. Name

| |
|---|
| (i) Order and/or higher taxon (for animals)<br>Prokaryote |
| (ii) Genus<br>Enterobacteriaceae: *Salmonella* |
| (iii) Species<br>*Salmonella enterica* |
| (iv) Subspecies<br>*Salmonella enterica* subspecies *enterica* serovar Typhi |
| (v) Strain<br>Ty2 |
| (vi) pathovar (biotype, ecotype, race, etc.)<br>Not known |
| (vii) common name<br>Attenuated *S. typhi* Ty2 (with deletions in 2 genes) |

3. Geographical distribution of the organism

| |
|---|
| (a) Indigenous to, or otherwise established in, the country where the notification is made:<br>Yes ☐ No ☐ Not known ☑ |
| (b) Indigenous to, or otherwise established in, other EC countries:<br>(i) Yes ☐<br>If *yes*, indicate the type of ecosystem in which it is found:<br>Atlantic ☐<br>Mediterranean ☐<br>Boreal ☐<br>Alpine ☐<br>Continental ☐<br>Macaronesian ☐<br>(ii) No ☐<br>(iii) Not known ☑ |
| (c) Is it frequently used in the country where the notification is made?<br>Yes ☐ No ☑ |
| (d) Is it frequently kept in the country where the notification is made?<br>Yes ☐ No ☑ |

4. Natural habitat of the organism

| |
|---|
| (a) If the organism is a microorganism<br>Water ☐<br>soil, free-living ☐<br>soil in association with plant-root systems ☐<br>in association with plant leaf/stem systems ☐<br>in association with animals ☐<br>other (specify): **Humans** |
| (b) If the organism is an animal: natural habitat or usual agroecosystem: |

5. (a)Detection techniques

| |
|---|
| Culture |

5. (b)Identification techniques

| |
|---|
| API Kit<br>PCR |

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

| Yes ☑ | No ☐ |
|---|---|
| If yes, specify: Public Health (Control of Disease) Act 1984<br>Public Health (Infectious Diseases) Regulations 1988<br>Control of Substances Hazardous to Health Regulations 1999<br>Categorisation of biological agents according to hazard and categories of containment (Second Supplement to fourth edition 1995) 2000 | |

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

| Yes ☐ | No ☑ | Not known ☐ |
|---|---|---|
| If yes: | | |
| (a) to which of the following organisms: | humans ☐<br>animals ☐<br>plants ☐<br>other ☐ | |

| | |
|---|---|
| (b) | give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC |

8. Information concerning reproduction

| |
|---|
| (a) Generation time in natural ecosystems:<br>*S. typhi* is human specific, the generation time *in vitro* is approximately 40 minutes. |
| (b) Generation time in the ecosystem where the release will take place:<br>The immunotherapy could be released into the Greater London sewage system following administration to humans. Wild type *S. typhi* does not persist or replicate in chlorinated water, in fact chlorination of water has had a major impact in eradicating typhoid fever as an endemic disease in countries such as the US.<br><br>Data is available to show that the recipient strain and the GMO cannot replicate or persist in tap water, confirming that the strain does not have a survival advantage in this environmental niche. |
| (c) Way of reproduction: Sexual ☐ Asexual ☑ |
| (d) Factors affecting reproduction:<br>The recipient is highly attenuated and can only undergo very limited rounds of replication without specific nutrient supplements that are not available *in vivo*. |

9. Survivability

| | |
|---|---|
| (a) Ability to form structures enhancing survival or dormancy: | |
| (i)endospores | ☐ |
| (ii) cysts | ☐ |
| (iii) sclerotia | ☐ |
| (iv) asexual spores (fungi) | ☐ |
| (v) sexual spores (fungi) | ☐ |
| (vi) eggs | ☐ |
| (vii) pupae | ☐ |
| (viii) larvae | ☐ |
| (ix) other, please specify: | **Not known** |

| |
|---|
| (b) Relevant factors affecting survivability: |

10. (a) Ways of dissemination

| |
|---|
| Faecal shedding. |

10. (b) Factors affecting dissemination

| |
|---|
| The survival times of the recipient strain in US tap water, soil and on surfaces are short. |

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

| |
|---|
| A DDX clinical study for the recipient strain was given permission by the SGHMS GMSC committee as follows:<br>SGHMS GMSC GM Number: 99.01<br>Organism: Group 1<br>Project: Type A (small scale, research)<br>This would have gone to HSE ACGM as part of the cumulative Annual Return. |

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Type of the genetic modification

| | |
|---|---|
| (i) Insertion of genetic material | ☑ |
| (ii) Deletion of genetic material | ☐ |
| (iii) Base substitution | ☐ |
| (iv) Cell fusion | ☐ |
| (v) Other, please specify | |

2. Intended outcome of the genetic modification

An *in vivo* regulated promoter fused to a Hepatitis B virus antigen gene has been inserted into the gene A deletion mutation of the recipient strain. The insertion results in the intracellular expression of the antigen. When delivered to humans, the GMO is intended to generate protective immune responses against the Hepatitis B virus antigen.

3. (a)Has a vector been used in the process of modification?

| Yes ☑ | No ☐ |
|---|---|
| If no, go straight to question 5. | |

3. (b)If yes, is the vector wholly or partially present in the modified organism?

| Yes ☐ | No ☑ |
|---|---|
| If no, go straight to question 5. | |

4. If the answer to 3 (b) is yes, supply the following information

| | |
|---|---|
| (a) Type of vector | |
| plasmid | ☐ |
| bacteriophage | ☐ |
| virus | ☐ |
| cosmid | ☐ |
| transposable element | ☐ |
| other, please specify | |

| |
|---|
| |
| (b) Identity of the vector |
| (c) Host range of the vector |

| |
|---|
| e(d) Presence in the vector of sequences giving a selectable or identifiable phenotype<br>Yes ☐ No ☐<br>Antibiotic resistance ☐<br><br>Other, specify<br>Indication of which antibiotic resistance gene is inserted |
| (e) Constituent fragments of the vector |
| (f) Method for introducing the vector into the recipient organism<br>(i) transformation ☐<br>(ii) electroporation ☐<br>(iii) macroinjection ☐<br>(iv) microinjection ☐<br>(v) infection ☐<br>(vi) other, please specify |

5. <u>If the answer to question B.3 (a) and (b) is no, what was the method used in the process of modification</u>?

| | |
|---|---|
| (i) transformation | ☐ |
| (ii) microinjection | ☐ |
| (iii) microencapsulation | ☐ |
| (iv) macroinjection | ☐ |
| (v) other, please specify | **Electroporation** |

6. Information on the insert

| |
|---|
| (a) Composition of the insert<br>*In vivo* regulated promoter fused to a Hepatitis B virus antigen gene. |
| (b) Source of each constituent part of the insert<br>*In vivo* regulated promoter: *Salmonella typhimurium* TML, amplified from chromosomal DNA.<br>Antigen gene – The gene was synthetically synthetised. The antigen gene sequence |
| (c) Intended function of each constituent part of the insert in the GMO<br>The *in vivo* inducible promoter is used to drive intracellular expression of the antigen. The expression of the antigen in the GMO is intended to generate a protective immune response in humans against the antigen. |
| (d) Location of the insert in the host organism<br>- on a free plasmid ☐<br>- integrated in the chromosome ☑<br>- other, please specify |
| (e) Does the insert contain parts whose product or function are not known?<br>Yes ☐ No ☑<br>If yes, please specify |

D. INFORMATION ON THE ORGANISM(S) FROM WHICH THE INSERT IS DERIVED (DONOR)

1. Indicate whether it is a:

| | | |
|---|---|---|
| Viroid | ☐ | |
| RNA virus | ☐ | |
| DNA virus | ☐ | |
| bacterium | ☑ | |
| fungus | ☐ | |
| animal | ☐ | |
| - mammals | ☐ | |
| - insect | ☐ | |
| - fish | ☐ | |
| - other animal | ☐ | (please specify phylum, class) |
| other, please specify | | |

2. Complete name

| |
|---|
| (i) order and/or higher taxon (for animals)<br>Prokaryote |
| (ii) family name (for plants)<br>Enterobacteriaceae |
| (iii) genus<br>*Salmonella* |
| (iv) species<br>*Salmonella enterica* |
| (v) subspecies<br>Salmonella enterica subspecies enterica serovar typhimurium |
| (vi) strain<br>*S. typhimurium* TML |
| (vii) cultivar/breeding line<br>N/A |
| (viii) pathovar<br>N/A |
| (ix) common name<br>*S. typhimurium* TML |

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

| Yes ☑ | No ☐ | Not known ☐ |
|---|---|---|
| If *yes*, please specify the following<br>(a) to which of the following organisms? Humans ☑<br>animals ☑<br>plants ☐<br>other ☐ | | |
| (b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?<br>Yes ☐ No ☑ Not known ☐ | | |
| If yes, give the relevant information under Annex III A, point II (A), 11(d): | | |

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks related to exposure to biological agents at work?

| Yes ☑ | No ☐ |
|---|---|
| If yes, please specify<br>Public Health (Control of Disease) Act 1984<br>Public Health (Infectious Diseases) Regulations 1988<br>Control of Substances Hazardous to Health Regulations 1999<br>Categorisation of biological agents according to hazard and categories of containment (Second Supplement to fourth edition 1995) 2000 | |

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

| Yes ☐ | No ☐ | Not known ☑ |
|---|---|---|

E. INFORMATION RELATING TO THE GENETICALLY MODIFIED ORGANISM

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

| |
|---|
| (a) Is the GMO different from the recipient as far as *survivability* is concerned?<br>Yes ☐ No ☑ Not known ☐<br>Please specify |
| (b) Is the GMO in any way different from the recipient as far as mode and/or rate of *reproduction* is concerned?<br>Yes ☐ No ☑ Not known ☐<br>Please specify |
| (c) Is the GMO in any way different from the recipient as far as *dissemination* is concerned?<br>Yes ☐ No ☑ Not known ☐<br>Please specify |
| (d) Is the GMO in any way different from the recipient as far as *pathogenicity* is concerned?<br>Yes ☐ No ☑ Not known ☐<br>Please specify |

2. Genetic stability of the genetically modified organism

| |
|---|
| PCR evidence suggests that the gene A and gene B deletion mutations and the promoter- gene fusion inserted into the chromosome of the GMO are stable over at least 10 days of passaging. Not only are the genetic modifications of the GMO stable over this period, but the phenotypic characteristics exhibited by possessing a deleted gene A mutation also remain the same (ie auxotrophic requirements). Genetic stability during manufacture of the GMO is demonstrated for each batch. |

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

| | | |
|---|---|---|
| Yes ☐ | No ☑ | Not known ☐ |
| (a) to which of the following organisms?: | humans | ☐ |
| | animals | ☐ |
| | plants | ☐ |
| | other | ☐ |
| (b) give the relevant information specified under Annex III A, point II (A) (11) (d) and II (C) (2) (i) | | |

4. Description of identification and detection methods

| |
|---|
| (a) Techniques used to detect the GMO in the environment<br>Culture. |
| (b) Techniques used to identify the GMO<br>API kits.<br>Agglutination for GMO specific antigens.<br>PCR. |

F. INFORMATION RELATING TO THE RELEASE

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

| |
|---|
| Clinical study to determine safety and efficacy of the GMO in humans. |

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

| Yes ☑ | No ☐ |
|---|---|
| If yes, please specify<br>The GMO is human specific. | |

3. Information concerning the release and the surrounding area

| |
|---|
| (a) Geographical location (administrative region and where appropriate grid reference):<br>The release site will be BIBRA International Ltd, Carshalton, Surrey SM5 4DS (in the London Borough of Sutton). Grid reference TQ275621. As a consequence of release the GMO may be released into the UK sewage system. This will be primarily in the Greater London area. The UK sewage treatment system is designed to eliminate bacteria, including *Salmonella* (information supplied by Thames Water, process Quality, Cross Ness S.T.W., S.E.29AQ). |
| (b) Size of the site ($m^2$): The facility for administration of the GMO to volunteers occupies an entire floor of one building at BIBRA International Ltd.<br>(i) actual release site ($m^2$): See 3a above.<br>(ii) wider release area ($m^2$): Greater London. |
| (c) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:<br>The Oaks park is within 1000 meters of the site. |
| (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO.<br>Not Applicable. The organism is restricted in its host range to humans. |

4. Method and amount of release

| |
|---|
| (a) Quantities of GMOs to be released:<br>Thirty six volunteers will receive up to $10^9$ colony forming units (CFUs) of the GMO on two occasions. Therefore the maximum number of CFUs administered will be $7.2 \times 10^{10}$. No replication or multiplication of the organism prior to elimination of the organism from the body is expected to occur because of the attenuating mutations that have been introduced. Most of the organisms administered are ulikely 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. Previous clinical studies with these types of attenuated Salmonella strains have demonstrated that only several thousand organisms are shed from volunteers when doses of $5 \times 10^7$ CFU are administered. The GMO is unlikely to survive outside the human host. |
| (b) Duration of the operation:<br>A defined period between April 2003 – June 2004. |

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

The GMO vaccine (in a liquid formulation) will be administered in a clinical room that has a level of containment equivalent to a category 2 Laboratory. The room has separate hand-washing facilities. All clinical waste, including syringes, tissues, disposable clothing and other personal protective equipment and all items that may have been in contact with the immunotherapy, is contained within sealed bins until disposal by autoclaving and incineration. Separate sanitary facilities are available in close proximity and are separate to those for general use by employees. Only those employees registered by the local Advisory Committee of Genetic Manipulation are permitted to enter the Unit during the study days. This facility has been inspected and complies with the HSE and approved for the Contained Use of category 2 pathogens and GMOs.

Following immunisation, volunteers will be housed within the Unit for 6 hours. 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 these facilities directly enters the Public Sewers that are capable of containing and inactivating the GMO. After the volunteers leave the clinical site, faeces will also enter the Public Sewers. Subjects 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.

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

| |
|---|
| Environmental conditions will be those of the Greater London sewage system. |

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release

| |
|---|
| This GMO has not previously been released. |

G. INTERACTIONS OF THE GMO WITH THE ENVIRONMENT AND POTENTIAL IMPACT ON THE ENVIRONMENT, IF SIGNIFICANTLY DIFFERENT FROM THE RECIPIENT OR PARENT ORGANISM

1. Name of target organisms (if applicable)

| |
|---|
| (i) order and/or higher taxon (for animals)<br>The target organism is humans. |
| (ii) family name (for plants) |
| (iii) genus |
| (iv) species |
| (v) subspecies |
| (vi) strain |
| (vii) cultivar/breeding line |

| |
|---|
| (viii) pathovar |
| (ix) common name |

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

| |
|---|
| Human volunteers ingest the GMO orally. The organisms will reach the small intestine and will be taken up by specialised antigen presenting cells in which the Hepatitis B virus antigen will be expressed. Consequently the host is expected to elicit an immune response against the GMO and the inserted Hepatitis B virus antigen. |

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

| |
|---|
| A concern with live attenuated vaccines is that there is faecal transmission from the volunteers to non-target hosts. However strict exclusion criteria for the trial have been set to minimize the risk of transmission of the GMO to these groups. Individuals who work as commercial food handlers, who are health care workers with direct contact with patients, who have household contacts with immuno-compromised individuals, pregnant women or the young or elderly will all be excluded from the trial. As mentioned above, 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 |

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

| Yes ☐ | No ☑ | Not known ☐ |
|---|---|---|
| Please give details. | | |

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established.

| |
|---|
| The organism will disseminate into the UK sewage system. The UK sewage treatment system is designed to eliminate bacteria, including *Salmonella* (information supplied by Thames Water, Process Quality, Cross Ness S.T.W., S.E.29AQ). |

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

| |
|---|
| (i) order and/or higher taxon (for animals)<br>Information not available. |
| (ii) family name (for plants) |
| (iii) genus |
| (iv) species |
| (v) subspecies |
| (vi) strain |
| (vii) cultivar/breeding line |

| |
|---|
| (viii) pathovar |
| (ix) common name |

7. Likelihood of genetic exchange in vivo

| |
|---|
| (a) from the GMO to other organisms in the release ecosystem:<br>Very low since the GMO does not contain any plasmids. |
| (b) from other organisms to the GMO:<br>The only way that the GMO could acquire the deleted sequences and regain a virulent phenotype is by plasmid transfer or other methods of genetic exchange (conjunction, transduction, re-assortment) with a donor organism. However this is |
| (c) likely consequences of gene transfer:<br><br>It is highly unlikely that a ΔA ΔB mutant could regain a virulent phenotype by acquisition of both the wild type genes A and B from the gut flora following oral immunisation , particularly as normal gut flora do not contain gene B. Many of the bacteria present in the normal gut flora (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*, non-pathogenic *E. coli* and others) contain A genes. However, ΔA mutants have been administered to humans in a number of clinical trials and reversion to virulence has not been reported.<br><br>Since *S. typhi* demonstrates little genetic variation worldwide, appearing almost as clones, its propensity for genetic exchange is extremely low.<br>Organisms that could potentially donate a wild type gene B to the GMO are restricted to those containing a functional gene B such as *Yersinia*, *Shigella*, Enteropathogenic *E. coli*, *Pseudomonas aeruginosa* and *Chlamydia*. However none of these organisms are part of the normal gut flora. Even if a volunteer had a sub-clinical infection with one of these organisms, it is unlikely that they could complement the gene B deletion, since gene B shows maximally only 30% homology between species. |

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in simulated natural environments (e.g. microcosms, etc.):

| |
|---|
| Information not available. |

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

| |
|---|
| Not known. |

H. INFORMATION RELATING TO MONITORING

1. Methods for monitoring the GMOs

Public Water Supply Companies have in place mechanisms to quantify the coliform count of the Public Mains Water.

Release of the GMOs in the faeces of vaccinated subjects will be monitored by culturing stool samples for *Salmonella* on specific days throughout the duration of the trial. If the cultures are positive beyond day 7 appropriate antibiotics will be administered. Stool testing will be continued until two consecutive negative results have been obtained. The released GMOs can be identified by standard microbiological means (eg PCR) in samples of water or in clinical samples from symptomatic and asymptomatic individuals (stool, blood or urine). We have successfully identified the recipient organism in clinical samples obtained from vaccinated subjects using routine microbiological practice.

2. Methods for monitoring ecosystem effects

Public water supply companies have in place mechanisms to quantify the coliform count of the public mains water.

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

The released GMO can be uniquely identified by genetic analysis (PCR) to identify the presence of the inserted gene and the deletions.

If so required the GMOs from stool samples will be plated out on selective media, and a PCR reaction will be carried out on a colony using optimal temperature and concentration of primers.

| **Test** | **Specification** |
|---|---|
| PCR to confirm gene B deletion | Presence of a 0.7 kb PCR fragment |
| PCR to confirm promoter-gene fusion in the gene A deletion. | Presence of promoter 0.45 kb. Presence of promoter and inserted gene 0.45 kb + 0.38 kb **2 PCR reactions**. |

When the GMO is plated out on selective media and a PCR reaction is carried out on a colony using the optimal temperature and concentration of primers, then the specificity of the technique is 100%.

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

Not applicable.

5. Duration of the monitoring

This will take place in the defined clinical study period between April 2003 and June 2004.

6. Frequency of the monitoring

| |
|---|
| Shedding of the GMOs in the faeces of vaccinated subjects will be monitored by culturing stool samples for *Salmonella* on specific days throughout the duration of the trial. If the cultures are positive beyond day 7 testing will be continued until two consecutive negative results have been obtained, and then as scheduled. In case of persistent shedding in the stool the Clinical Investigator will use his discretion regarding the administration of antibiotics. |

I. INFORMATION ON POST-RELEASE AND WASTE TREATMENT

1. Post-release treatment of the site

The facility will receive surface disinfection of hard surfaces. Disposable items will be incinerated.

2. Post-release treatment of the GMOs

There will be no post-release treatment of the GMOs. As a consequence of release, the GMOs may disseminate into the Greater London sewage system. The Greater London sewage treatment system is designed to eliminate bacteria, including *Salmonella* (information supplied by Thames Water, Process Quality, Cross Ness S.T.W., S.E.29AQ).

3. (a)Type and amount of waste generated

1. Laboratory waste: plastic ware, liquid reagents and microbial cultures
2. Clinical waste: faeces, urine and blood samples.
3. Miscellaneous waste: disposable clothing, tissues.
4. Scale of waste: small.

3. (b)Treatment of waste

A. Laboratory waste: Solid laboratory waste will be subjected to 10 minutes of steam infusion before autoclaving at 125°C for 30 minutes (throughout the load) in validated autoclaves on site, and then sealed within rigid containers for removal and incineration by White Rose Environmental.

Liquid waste will be inactivated by hypochlorite solution and then, depending on volume and potential contamination, either disposed of into the sewerage system or run onto sawdust and autoclaved before incineration.

B. Clinical waste: If clinical waste contains the GMO, it is likely to be present at only very low levels. Faecal and urine samples collected into lidded, plastic containers for analysis will be sealed into plastic bags within a containment hood and autoclaved and incinerated as described above. This will reduce the likelihood of direct transmission to staff handling the material. Blood samples will be similarly treated.

C. Miscellaneous waste: All items that have the potential for contamination, such as disposable clothing, tissues etc will be placed in sealed containers prior to autoclaving and incineration.

J. INFORMATION ON EMERGENCY RESPONSE PLANS

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

Transmission of the GMO would be solely human to human with no animal, plant or insect vector. All control measures would be to identify a person carrying the GMO or susceptible to infection with the GMO. The volunteers will be kept in the vaccination unit for observation for 48 hours after administration. If any volunteers vomit this will be treated as a biological hazard and a suitable disinfectant will be used to inactivate the hazard, however since the GMO is severely attenuated it will not survive outside the human host.

The GMO is sensitive to ciprofloxacin antibiotic which 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 (eg 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. Transmission within the environment is readily controlled by chlorination of drinking water, sewage treatment processes and disinfection of hard surfaces by standard disinfectants.

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

Transmission within the environment is readily controlled by chlorination of drinking water, sewage treatment processes and disinfection of hard surfaces by standard disinfectants (eg hypochlorite solution “Virkon”).

As a consequence of release the GMO may be released into the Greater London sewage system.

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

| |
|---|
| Treatment with hypochlorite solution, incineration or autoclaving at or above 121°C for 15 minutes are all effective means for decontamination of exposed items. |

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

The clinical trial procedures ensure close clinical, haematological, biochemical, physiological and microbiological monitoring of immunised volunteers for any adverse effects.

The volunteers will be kept in the vaccination unit, for observation, for 48 hours after administration. If any of the volunteers' vomit this will be treated as a biological hazard and a suitable disinfectant will be used to inactivate the hazard, however since the GMO is severely attenuated it will not survive outside of the human host.

There will be treatment of any immunised volunteer with antibiotics should an adverse effect be observed owing to the vaccine strain. The antibiotics will be administered at the Investigators discretion. The Principal Investigator is a Medical Practitioner on the GMC Specialist Register for Communicable Diseases and General (Internal) Medicine and has on-site facilities to immediately admit to hospital, under quarantine if necessary, any volunteer manifesting an undesirable effect. The site has a purpose built Infectious Diseases Clinical Facility with ability to admit cases infected with pathogens up to and including ACDP Category 4. There are also Intensive Care and other facilities on site.

The Principal Investigator works closely with the PHLS Monitoring Centre on-site, providing the opportunity to rapidly identify any unusual disease activity suggesting an undesirable spread of the organism or unexpected undesirable side effect of the GMO. This PHLS Centre covers most of the region in which the release will take place and is therefore in a good position to detect any undesirable effects in the local community. The GMO is human specific and will not survive in the environment. Therefore no undesirable effect from the GMO is anticipated.