

Part B: Information about the release application to be included on the Public Register

B1 The name and address of the applicant

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B2 A general description of the genetically modified organisms in relation to which the application is being made

Description of the GMO

The GMO is an isogenic mutant of a wild-type *Salmonella* Typhi (S.Typhi) Quail's strain. The purpose of the genetic modification is to construct a modified S. Typhi Quail's strain carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, to generate a typhoid toxin-deficient S. Typhi Quail's strain (SB6000). The GMO (SB6000) will be used to investigate the role of typhoid toxin in the pathogenesis of enteric fever in a controlled human infection model.

Description of the application

The primary objective of the study is to compare the proportion of participants developing clinical or microbiologically proven typhoid infection following oral challenge with the wild type S. Typhi Quail's strain with participants challenged with a GMO typhoid toxin-deficient isogenic mutant of S. Typhi Quail's strain SB6000. Typhoid fever is caused by infection with the bacterium S. Typhi. The GMO to be released is intended for use in healthy adults aged 18-60 years. The Oxford Vaccine Group (University of Oxford, UK) has been undertaking controlled human challenge studies using S. Typhi and S. Paratyphi A since 2010.

Converging lines of evidence suggest that typhoid toxin plays a central role in the pathogenesis of typhoid fever, which may account for the human-restriction properties of this pathogen as well as being a strongly immunogenic antigen following natural infection. These data raise the intriguing possibility that typhoid toxin could be a promising vaccine candidate for typhoidal *Salmonella*. The lack of a meaningful animal model has, however, hampered the ability to study the potential importance of typhoid toxin in the pathogenesis of typhoid fever. In this study we propose to utilise the human challenge model to further study host-pathogen interactions by investigating the role of the typhoid toxin, in

the pathogenesis of typhoid fever. This information will be used to inform vaccine design and development potentially influencing public health intervention strategies.

B3 The location at which the genetically modified organisms are proposed to be released

The address of the proposed site of release is: Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE. The national (OS) grid reference for the proposed site release is SP543060.

As a consequence of shedding through faecal material the GMO may be released into the sewage system in England and primarily within the Oxfordshire area. The sewage system is designed to contain and clear bacteria including *Salmonella*.

B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

The purpose of the study is to investigate the contribution of typhoid toxin to *S. Typhi* infection and typhoid fever using a human challenge model of infection. The typhoid toxin-deficient *S. Typhi* Quail's SB6000 strain will be evaluated in a healthy volunteer challenge model alongside the wild type *S. Typhi* Quail's strain. The primary objective of the study is to compare the proportion of participants developing clinical or microbiologically proven typhoid infection following oral challenge with the wild type *S. Typhi* Quail's strain with participants challenged with a typhoid toxin-deficient isogenic mutant of *S. Typhi* Quail's strain (named SB6000). The study will compare both the clinical and laboratory features following challenge with either wild-type *S. Typhi* Quail's strain or typhoid toxin-deficient isogenic GMO strain including a detailed investigation of the host response to infection.

B5 The intended dates of the release.

The exact timing of the release will be dependent on all study approvals being in place. We anticipate that clinical study will commence on the 1st of September 2016 and recruitment may take up to 12 months (until 31st of August 2017). A total of 40 participants will be enrolled to the study from within the Oxfordshire area. The GMO will be given orally to study participants who are likely to shed the organism in faeces/stool. This shedding constitutes the release of the GMO. The duration of the release (involving both oral challenge and the shedding phase of the study) is expected to be no longer than 18 months.

B6 The environmental risk assessment.

The GMO is an isogenic mutant of the wild type (parent) *S. Typhi* Quailles strain and has been designed and manufactured to GMP standard for use as an oral challenge agent within a controlled human infection model to be conducted at the Oxford Vaccine Group (University of Oxford, UK). The GMO will be administered orally to healthy volunteers recruited to the study who are likely to shed the organism in faeces/stool. This will constitute the release of the organism resulting in release into the sewage system. Normal basic hygiene precautions, namely the use of toilets and hand washing, are considered sufficient to prevent person to person transmission. The GMO is expected to be effectively contained and inactivated by usual sewage system processes.

Survival and Persistence Characteristics

To evaluate the survival and persistence characteristics of both the wild type parent strain (Quailles) and typhoid toxin-deficient isogenic mutant (SB6000) a series of experiments were conducted in river water, sea water and soil. In conclusion the data demonstrate that the GMO strain SB6000 did not persist for any longer than the wild type Quailles strain from which it is derived from. The data demonstrate that both strains survive only transiently in the environment.

River and sea water:

River water aliquots were inoculated with the *S. Typhi* wild type (WT) and SB6000 GMO strains. All aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the viable cell count (VCC) of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of water. The rate of decline over a period of 68 days was similar for both the WT and GMO (SB6000) strains (Figure 1).

Sea water aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the VCC of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of water. Neither the GMO (SB6000) nor the WT *S. Typhi* persisted beyond day 51 (Figure 1).

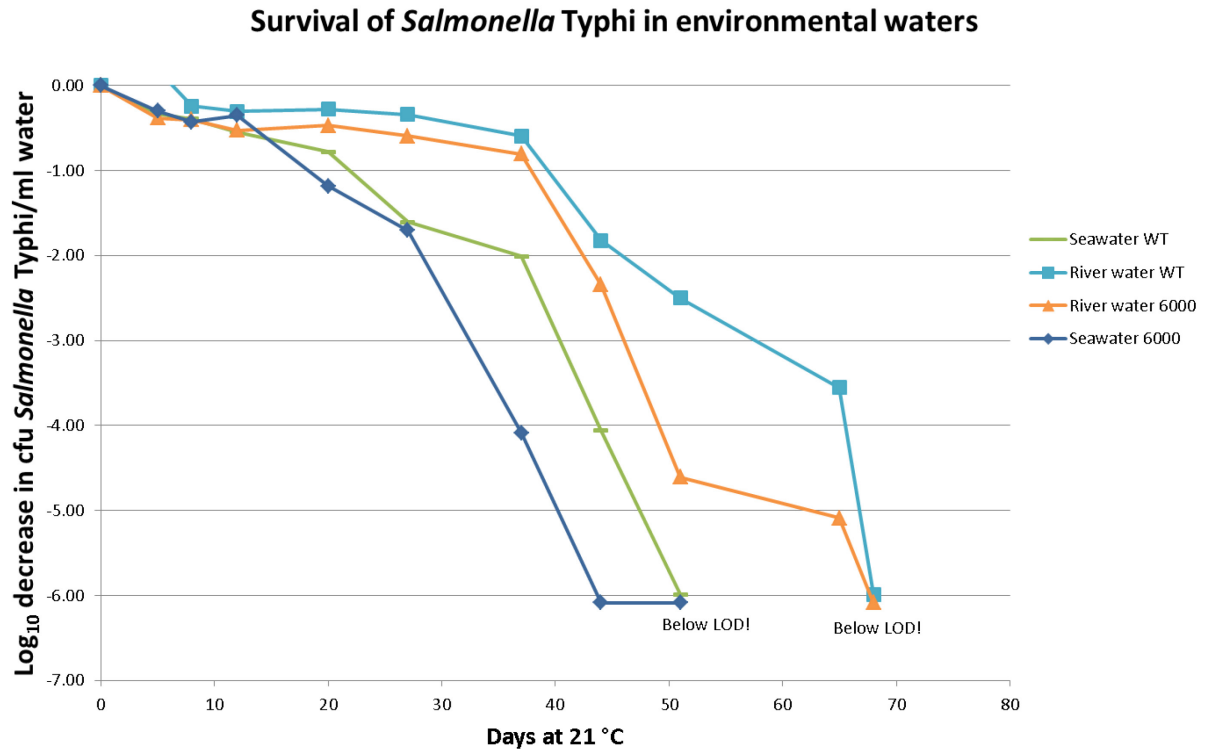


Figure 1. Survival of survival of *Salmonella* Typhi WT strain Quailles and its SB6000 GMO derivative in river and sea water.

Soil:

Aliquots of top soil were inoculated with the *S. Typhi* WT or SB6000 GMO strains. All aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the viable cell count of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of soil suspension. It was concluded from this study that the GMO survived for only a limited time in soil. The GMO persisted for no longer than 44 days at the highest inoculum level tested and no longer than 37 days at the lower inoculum level (Figure 2).

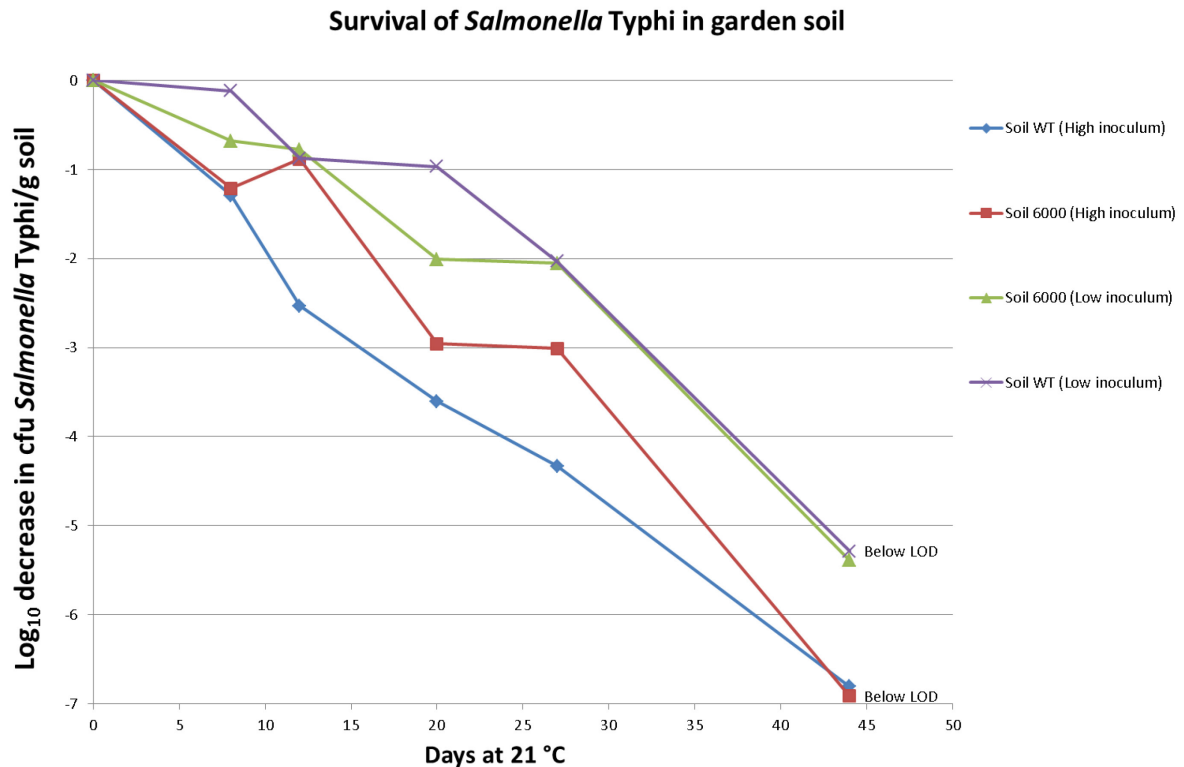


Figure 2. Survival of *Salmonella* Typhi WT strain Quailles and its SB6000 GMO derivative in garden soil.

Human Host Specificity

Wild type *S. Typhi* has no known animal reservoir (human restricted pathogen) and the GMO will not have a selective or survival advantage. The potential for genetic exchange with any other organism in the environment is extremely low given that the GMO does not contain any plasmids or antibiotic resistance markers. The GMO is not expected to persist in study participants following the completion of 14 days of antibiotic treatment.

Despite extensive studies, performed over a century in numerous animal species, it has not been possible to establish an infection with *Salmonella* Typhi that resembles typhoid or indeed any long term persistent infection other than in higher primates. *S. Typhi* can infect higher primates such as the chimpanzee but again, the disease does not fully replicate human typhoid, in part because the typhoid toxin receptor is not expressed in this species. Intensive efforts were made in the past several decades to genetically manipulate *S. Typhi* to become infective for other species, including the mouse, but these all failed.

S. Typhi remained avirulent in mice even if large regions of the genome of murine pathogenic *Salmonella* are mobilised into *S. Typhi*. By examining the genome of *S. Typhi* we now have a scientific explanation for these data in that (a) the Typhi genome harbours upwards of 300+ so called pseudogenes (inactivated genes), many of which are likely to be required for infectivity in other hosts (b) Typhi has acquired a number of different gene sets, including typhoid toxin, Typhi IV pili and Vi

antigen that facilitate infection in humans. Against this background it is hard to formulate a hypothesis that deletion of the typhoid toxin genes would enhance the ability of *S. Typhi* to infect other species, even if individuals were immunocompromised.

Sample Size

The analyses for the primary endpoint of the study will be descriptive in nature and will not include formal hypothesis testing. A total of 40 participants will be randomised 1:1 to receive challenge with either wild type *Salmonella* Typhi Quailles strain (WT) or the typhoid toxin-deficient isogenic mutant of *S. Typhi* Quailles strain SB6000 (a minimum sample size of 20 participants in each group will be required). It is anticipated that the attack rate following challenge with the SB6000 strain will be reduced compared to the WT strain. Assuming an attack rate of 65% following WT strain (as observed in previous studies) and 50% attack rate following SB6000 strain, and accounting for 10% drop out, 20 participants in each group will lead to 95% confidence intervals for attack rate of 41% to 85% in the group randomised to WT strain and 27% to 73% in the group randomised to SB6000 strain.

For the purpose of the release, the GMO will be given to a minimum of 20 participants at a single site in Oxford (no other sites will be enrolled to participate in this study). Additional participants may receive the GMO to account for participant withdrawals before the completion of antibiotic treatment period of the study in order to achieve a minimum of 20 participants in each *S. Typhi* challenge group. The study participants will be healthy adult volunteers aged 18-60 years. During the initial post-challenge phase the study participants must be resident within Oxford (up to 28 days post challenge). Stool clearance will be confirmed following 3 negative stool samples provided at least 48 hours apart following completion of a 14 day course of antibiotics. We are unable to provide information on the safety of the GMO when administered to healthy subjects as this is the first application of the organism in a clinical study. The risk of both the GMO and wild-type strain to other humans is considered to be low and the risk to the environment is considered to be effectively zero.

B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

Methods and plans for monitoring the GMO

The health and safety of our study participants is of the utmost importance and will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the site study physicians as appropriate.

Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Time to onset of stool shedding - time from challenge (Hours/Days) to the first positive stool culture, excluding the first 24 hours following ingestion of challenge agent will be documented. Participants will be required to supply further stool samples until proven not to be shedding *S. Typhi*. Stool samples will be collected at least one week after completion of a 14 day course of antibiotics, until three successive stool samples

(collected at least 48 hours apart) are negative for *S. Typhi*. If persistent stool shedding occurs after completion of antibiotics, participants will be referred to the Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust.

Additionally, quantitative stool cultures or PCR may be performed to assess the burden of stool shedding. Isolates from stool samples will be stored frozen for future analysis, which may include phage typing or genetic sequencing.

Frequency of the monitoring

Monitoring will take place for the duration of the clinical study. The study is expected to commence on the 1st of September 2016 (pending all necessary approvals) and will run for approximately 18 months (with an expected completion date of March 2018). All study participants will have follow-up visits up to 1 year post challenge.

Following challenge with the GMO and wild type Quail strain participants will be monitored daily for the first 14 days post-challenge.

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

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

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

has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

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

Stringent precautions are in place to avoid the spread of the GMO from the study participant to others. Such spread has not been noted in previous studies conducted by the Oxford Vaccine Group using the wild type *S. Typhi* Quail's strain. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing). In view of the low infectivity of *S. Typhi* (without administration in sodium bicarbonate buffer) and the high standard of hygiene and sanitation in the UK, secondary transmission of the challenge strain to household or other close contacts is highly unlikely. It is acknowledged, however, that transmission within households can occur if the individual excreting *S. Typhi* fails to practice effective hand washing after defecation and is subsequently involved in uncooked food preparation. The participant will provide letters from the study team to close contacts including household contacts. Contacts will be offered the opportunity to be screened for *S. Typhi* infection, which will involve obtaining two stool samples 48-hours apart. If either stool culture of a household contact is positive, he/she will be referred to a Consultant in Infectious Diseases for appropriate antibiotic management and Public Health England will be informed.

Methods and plans for responding to an emergency

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

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

Participants will be issued with a Medic Alert-type card containing information including the antibiotic sensitivity of the *S. Typhi* strain (GMO and wild type Quail's)

strain), study doctor contact details and instruction for the research team to be contacted immediately in the event of illness/accident.

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

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