PART B: INFORMATION ABOUT THE RELEASE APPLICATION TO BE INCLUDED ON THE PUBLIC REGISTER

B1 The name and address of the applicant

Prokarium Ltd, London Biotechnology Innovation Centre, 2 Royal College St, London NW1 0NH.

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

In this application the genetically modified organisms to be released as part of this application are ZH9 and ZH9PA.

ZH9 is a genetically modified organism derived from a bacterial strain of Salmonella enterica subspecies enterica serovar Typhi strain Ty2 (abbreviated to S. Typhi Ty2). It has previously shown promise as a vaccine to protect against enteric fever caused by S. Typhi.

This strain contains deletions within certain genes (aroC and ssaV) as previously described in Application 10_R40_01 (15 Dec 2010). The modified strain is known as S. Typhi (Ty2 aroC- ssaV-) ZH9, abbreviated to ZH9. This strain was developed by Emergent Biosolutions as a single dose typhoid vaccine. Subsequently Emergent ceased this development and the technology was acquired by Prokarium Ltd., who are now developing the GMO in the present application.

The specific deletions within the above-named genes which results in attenuation (modifying the bacteria so they are unable to cause disease) to generate ZH9 are as follows: deletion of the aroC gene prevents the activity of an enzyme responsible for the production of proteins within the Salmonella bacteria. Deletion in the ssaV gene means that one of the systems required for survival and growth of the Salmonella in humans is unable to function. This therefore limits the ability of the Salmonella to replicate and spread throughout the body. These deletions act on different pathways, so providing an additional level of safety.

The ZH9PA strain to be administered in this study is a further modification of the ZH9 strain described above to make it express key protective antigens which may broaden the protection to also cover enteric fever caused by S Paratyphi A. The ZH9PA is modified as follows: 1) replacement of the H:d allele (variation of a gene) of the flagellin (protein structure which forms a tail like whip to allow the bacteria to move) with the H:a allele which is expressed by S. Paratyphi A; 2) deletion of a gene which codes for the conversion of abequose to tyvelose and changes the form of the LPS (carbohydrate outer coating of the bacterial cell) from the form expressed in S. Typhi to that expressed in S. Paratyphi A.

The experimental enteric fever vaccine (ZH9/ZH9PA) to be administered in this study is an equal mixture of the parental organism (ZH9) with the novel GMO (ZH9PA) formulated with excipients.

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

The genetically modified organisms are proposed to be released at: Simbec-Orion Clinical Pharmacology, Merthyr Tydfil Industrial Park, Cardiff Road, Merthyr Tydfil, CF48 4DR.

The national (OS) grid reference of the proposed site of release is SO 064033.

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 GMO will be administered to healthy participants in a Phase I placebo-controlled clinical study. The clinical protocol 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 investigate the safety of and immune responses to increasing doses of the vaccine, or placebo in healthy participants. Data collected from this study will support future development of the ZH9/ZH9PA oral enteric fever combination vaccine.

B5 The intended dates of the release.

The intended dates of release are between September 2019 and September 2021. The GMO is scheduled to be released on 3 occasions over a period of 42 days for each of the 3 study groups.

B6 The environmental risk assessment.

Due to the intended proposed site of release, it is considered that the risk to the environment as a result of the release is negligible. In this release the GMO will be excreted into the sewage system and subjected to normal sewage treatment processes, which are considered suitable for the treatment of wild type S. Typhi (since individuals infected with typhoid fever are not, in the UK, required to take any special precautions in the disposal of their stools).

There is a possibility that the GMO could enter other environments such as soil and water bodies. However this should only occur should a breach in the sewage system occur, or if faecal samples containing the GMO were disposed of via facilities that do not involve a mains sewage system. This is not the case at the proposed site of release.

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

There is a risk of the GMO being transmitted from participants to other individuals; however the following measures are in place to minimize this risk.
In this release, the GMO will be excreted directly into the sewage system and it is expected that it will be contained there to be subject to normal sewage processing treatments. It is expected, based on evaluation of shedding in previous clinical trials with the parent strain (ZH9), that the GMO will be shed by participants for no longer than 17 days post-dosing.

Strict exclusion criteria have been set for the trial to minimise the risk of transmission of the GMO, and in particular to minimise transmission to potentially

vulnerable groups such as immuno-compromised individuals, pregnant women or the very young. Participants will be instructed on how to maintain strict personal hygiene and proper hand washing will be taught and reinforced to minimise the risk of faecal-oral transmission. Additionally participants will be restricted with respect to travel outside of the UK until is confirmed via stool culture that participants are no longer shedding bacteria.

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

All clinical staff will be appropriately trained according to GCP and documented local procedures. Trial personnel in direct contact with participants and/or responsible for handling the GMO will use personal protective equipment (aprons and gloves) as appropriate.

Following each release event (i.e. each administration of a dose of GMO), the dosing area will be cleaned and disinfected.
In clinical areas, routine cleaning of surfaces will be performed according to local procedures using alcohol wipes.

Larger spillages will be treated with chlorine-releasing tablets 1.7g (Actichlor) in for 2 minutes, then cleared up, wearing appropriate personal protective equipment. The area will then be washed with hot water and detergent. In laboratory areas, surfaces (including floors and benches) will be decontaminated with 1% Virkon solution.

Minor surface contamination will be treated with 1% Virkon for 10 minutes. Larger spillages (including blood and body fluids) will be treated with Virkon granules for a minimum of 10 minutes.
Any equipment used for dosing will be cleaned and decontaminated (e.g. by autoclaving) or disposed of (e.g. by incineration), as appropriate. All disposable materials will be contained as appropriate in clearly labelled bags or sharps bins and disposed of as clinical waste according to local procedures. All disinfection, decontamination and disposal procedures will be performed wearing suitable personal protective equipment in accordance with documented local procedures including those for Infection Control.

The GMO will be administered in a designated room with separate hand washing facilities. Following dosing with the GMO, participants will stay at the Study Centre under observation for 4 hours. The waste from the sanitary facilities at the site enters directly into the public sewers which are capable of containing the organism.
After dosing, all surfaces will be disinfected according to documented local procedures. All clinical waste, including tissues, disposable clothing and other miscellaneous waste will be placed into biohazard bags contained within closed bins and disposed of as clinical waste according to local procedures.

If any of the participants vomit following administration of the GMO at the clinical site, this will be treated as a biological hazard. Suitable personal protective equipment and disinfectant will be used in the inactivation of the hazard. All resulting waste will be disposed of into sealed containers for autoclaving and

incineration, in accordance with local documented procedures for waste disposal and for the management of participants with vomiting and diarrhoea. However, since the GMO is severely attenuated it will not survive outside the human host. All study samples and specimen sample bags must be labelled with a 'Danger of Infection' label and transported in accordance with local documented procedures.

Contaminated areas may be 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.
Transmission of the organism within the environment is readily controlled by sewage treatment processes.

Participants in the clinical trial will have stool cultures performed at Day 70 (28 days after the last dose) to document the fact that they are no longer shedding the GMO. In the event that a positive stool culture is obtained, participants will be treated with a course of antibiotics previously shown to be effective at eradicating ZH9. Following completion of the course of antibiotics participants will be required to provide another stool sample on Day 84 to confirm that they are no longer shedding.