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

Data or results are requested from any previous releases of the GMO for which the applicant is seeking a consent, especially the results of monitoring and the effectiveness of any risk management procedures.

The GMO was previously released in accordance with the conditions applied to the consent for deliberate release of the GMO (application reference number 02/R37/2).

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 GMO was administered to the volunteers at BIBRA International Ltd, Carshalton, Surrey, UK. The immunotherapy was administered orally in a volume of sodium bicarbonate solution. 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.

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. Shedding of the GMO in the faeces of vaccinated subjects was closely monitored by culturing stool samples for \textit{Salmonella} on frequent occasions specified throughout the duration of the trial.

Overall the immunotherapy was well tolerated by all subjects and demonstrated a good safety profile. No serious adverse events related to the investigational product were reported and there were no unexpected adverse events. The majority of events were mild in severity. Nine subjects reported 18 moderate events post-vaccination and three subjects reported a total of 5 severe adverse events, all of which were assessed as unlikely to be related to the study medication. Post-vaccination events relating to fever were reported by 6 subjects and 2 of these events, one of moderate severity, were considered to be possibly related to the immunotherapy. However the events were generally mild and did not prevent the subjects continuing in the trial. The result of laboratory tests did not reveal any adverse events that appeared to be related to the immunotherapy. The GMO was not isolated from any of the blood or urine samples and, in-line with expectations, shedding did not occur in any subjects beyond 6 days after dosing. It was not necessary to administer antibiotics to any subject for a suspected \textit{S. typhi} (GMO-related) infection.
Stool samples were cultured on days 1, 2, 3, 4, 5, 6, 7, 11, 14 and 28 after vaccination to monitor for the presence of the GMO. Following administration of the first dose of immunotherapy there was little difference in faecal shedding profiles of subjects in the high (1 x 10^9 CFU) and low dose (1 x 10^8 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 10^8 CFU and 38 doses of 1 x 10^9 CFU of the immunotherapy were administered, a total of approximately 4 x 10^{10} CFU. No shedding was detected beyond 6 days after dosing and therefore release of the GMO into the Greater London sewage system occurred between the dates 27 August 2003 and 22 January 2004.

Further details of safety monitoring:

- 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 be 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.
PART A3: DETAILS OF PREVIOUS APPLICATION FOR RELEASE

One application has previously been made for deliberate release of this GMO.

The application reference number is 02/R37/2.

The application was dated 20 December 2002 and was made by Microscience Ltd (the company name changed to Emergent Europe Ltd in July 2005).

Consent was notified on 16 July 2003.

A report on the effects of the release was submitted on 06 February 2004. The report contained details of the shedding of the bacteria and the general health of the volunteers.