Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 126

Clinical trial of a genetically modified vaccine against Cholera

Applicant: PaxVax Australia Pty Ltd (PaxVax)

April 2014

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 126

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this clinical trial of a genetically modified (GM) vaccine. This clinical trial is a limited and controlled release application under the *Gene Technology Act 2000* (the Act). A science based Risk Assessment and Risk Management Plan (RARMP) for the application was prepared by the Regulator in accordance with requirements of the Act and corresponding state and territory legislation, and finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that the proposed clinical trial poses negligible risks to human health and safety and the environment.

The application

Application number	DIR 126
Applicant	PaxVax Australia Pty Ltd (PaxVax)
Project title	Clinical trial of a genetically modified vaccine against Cholera[1]
Parent organism	Cholera bacterium (*Vibrio cholerae*)
Introduced or modified genes and resulting modified traits	• deletion of *Cholera toxin A subunit* gene (*ctxA*) (loss of toxin expression - vaccine attenuation) • inactivation of *haemolysin A* gene (*hlyA*) (loss of toxin expression - vaccine attenuation) • insertion of mercury resistance operon (*mer*) from *Shigella flexneri* NR1 (selectable marker – mercury resistance)
Proposed locations	Clinical sites in Queensland, South Australia, Victoria and Western Australia
Proposed trial size	A maximum of 1000 volunteers covering different age groups are proposed to be enrolled for the clinical trial
Proposed trial dates	10 April 2014 – 30 June 2015
Primary purpose	To verify the effectiveness of the vaccine in producing an immune response against cholera

[1] The title of the licence application submitted by PaxVax is “Clinical development of a recombinant live oral cholera vaccine (PXVX0200)”.

The GM vaccine contains live genetically modified *V. cholerae* bacteria. Unmodified cholera bacteria produce a toxin containing 2 subunits (A and B) and haemolysin (a protein which can break open blood cells). The vaccine strain has been produced by deleting most of the toxic *A subunit* gene (*ctxA*) and inserting a mercury resistance operon (*mer*) into the *haemolysin* gene (*hlyA*). As a result of the genetic modification the GMOs cannot produce the A-subunit of the cholera toxin molecule or haemolysin. The non-active B-subunit of the cholera toxin molecule is still synthesised but this protein does not cause disease or toxicity on its own.

The *mer* operon was inserted into the *hlyA* gene to allow easy and rapid differentiation between the GM cholera vaccine strain, and the wild-type toxin producing *V. cholerae.* The *mer* operon does not encode proteins that can produce, store or sequester mercury. There is no mercury associated with the vaccine.

The clinical trial will involve oral administration of the vaccine to volunteers (both children and adults) in Australia. This trial will form part of a larger international study including trials in the USA and Canada. The purpose of these trials is to verify the effectiveness of the vaccine in producing an immune response against cholera. The Australian trial will take place in clinical facilities in Queensland, South Australia, Victoria and Western Australia. Once underway the trial is expected to be completed within approximately one year, depending upon the availability of volunteers.

Medicines and other therapeutic goods for sale in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods. The Therapeutic Goods Administration (TGA) is responsible for administering this legislation. This clinical trial must also be conducted in accordance with the relevant TGA requirements.

Risk assessment

The risk assessment concludes that risks to the health and safety of people, or the environment, from the proposed release are negligible.

The risk assessment process considered how the genetic modification and activities proposed to be conducted with the GMOs might lead to harm to people or the environment. Risks were characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application (including proposed limits and controls), relevant previous approvals and current scientific and technical knowledge. Both the potential short and long term harms were considered.

Credible pathways to potential harm that were considered included whether or not expression of the introduced genes or changes in gene expression due to gene deletions could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM Cholera bacterium (*V. cholera*); or produce unintended changes in bacterial characteristics. The opportunity for unintended exposure to the vaccine or the GM bacteria it contains, and for gene flow to other organisms was also considered. No new risks to people or the environment were identified from the advice received on the consultation RARMP.

The principal reasons for the conclusion of negligible risks are that the proposed limits and controls effectively contain the GMO and its genetic material and minimise exposure; the introduced genetic modifications are unlikely to cause harm to people or the environment; and genes similar to the introduced genes are common in the environment.

Risk management plan

The risk management plan concludes that risks posed by the proposed dealings can be managed so as to protect people and the environment by imposing conditions on the release.

Risk management is used to control or mitigate risk. The risk management plan evaluates and treats substantive risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, as this is a limited and controlled release, the licence includes limits on the size, locations and duration of the release, as well as controls including administration of the GM vaccine by trained staff, exclusion of individuals who could be at risk of adverse effects, fully informing volunteers participating in the trial, appropriate containment and waste disposal provisions at the clinical site, destroying GM vaccine not required for further studies and transporting the GM vaccine in accordance with the Regulator's transport guidelines or other specific conditions.

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Abbreviations

APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
CCI	Confidential Commercial Information under section 185 of the *Gene Technology Act 2000*
CFU	Colony forming unit
CTN	Clinical Trial Notification
CTX	Clinical Trial Exemption
Ctx	Cholera toxin
ctx	Cholera toxin genes/operon
ctxA	Gene coding for the A subunit of the cholera toxin
ctxAB	Genes coding for the entire cholera toxin
ctxB	Gene coding for the B subunit of the cholera toxin
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
FSANZ	Food Standards Australia New Zealand
GMAC	Genetic Manipulation Advisory Committee
GM	Genetically modified
GMO	Genetically modified organism
HGT	Horizontal gene transfer
hlyA	Haemolysin gene
HREC	Human Research Ethics Committee
HSNO Act	New Zealand's *Hazardous Substances and New Organisms Act 1996*.
HV	Healthy Volunteers
ICEs	Integrative and Conjugative Elements
ICH-GCP	The international conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, guidelines for good clinical practice
kb	Kilobase pair of DNA
mer	Mercury resistance genes/operon from *Shigella flexneri*
NHMRC	National Health and Medical Research Council
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
OGTR	Office of the Gene Technology Regulator
PXVX0200	Genetically modified vaccine against cholera containing vaccine strain *V. cholerae* CVD 103-HgR
QLD	Queensland
RARMP	Risk Assessment and Risk Management Plan
S. flexneri	*Shigella flexneri* (bacterial species that causes bacillary dysentery)
TCP	toxin co-regulated pili
tet	Tetracycline resistance gene
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*

the Regulator	Gene Technology Regulator
the Regulations	Gene Technology Regulations 2001
trial participant	a person who receives the GMO as a vaccine
USA	United States of America
V. cholerae	*Vibrio cholerae* (bacterial species that causes cholera)
WA	Western Australia
WHO	World Health Organisation

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

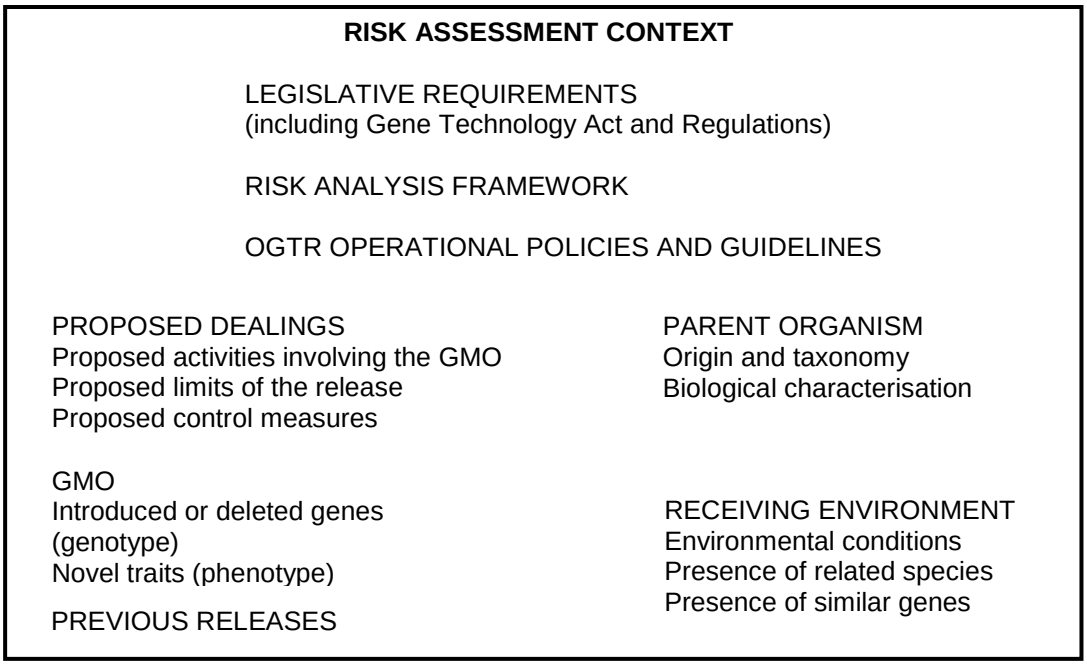

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. In accordance with section 50A of the Act, this application is considered to be a limited and controlled release application, as its principal purpose is to enable the applicant to conduct experiments and the applicant has proposed limits on the size, locations and duration of the release, as well as controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. Therefore, the Gene Technology Regulator (the Regulator) was not required to consult with prescribed experts, agencies and authorities before preparation of the Risk Assessment and Risk Management Plan (RARMP; see section 50 of the Act).

5. Section 51 of the Act and regulation 9A of the Regulations outline the matters the Regulator must take into account in preparing a RARMP.

6. Section 52 of the Act requires the Regulator to seek comment on the RARMP from the States and Territories, the Gene Technology Technical Advisory Committee, Commonwealth

authorities or agencies prescribed in the Regulations, the Minister for the Environment, relevant local council(s), and the public. The advice from the prescribed experts, agencies and authorities, and how it was taken into account, is summarised in Appendix A. A summary of the public submissions received and their considerations are summarised in Appendix B.

7. The *Risk Analysis Framework* (OGTR 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements. Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.

Section 3 Scope and boundaries

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods. The Therapeutic Goods Administration (TGA) is responsible for administering the provisions of this legislation. Clinical trials of therapeutic products that are experimental and under development, prior to a full evaluation and assessment, are also regulated by the TGA through the Clinical Trial Exemption (CTX) scheme or the Clinical Trial Notification (CTN) scheme.

9. For clinical trials, TGA has the regulatory role in the supply of the unapproved therapeutic product. In terms of risk to the individuals participating in a clinical trial, TGA (as the regulator), the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) all have roles in ensuring the participants' safety under the *Therapeutic Goods Act 1989*. However, where the trial involves a GMO, authorisation is also required under gene technology legislation. In order to avoid duplication of regulatory oversight, as risks to trial participants are addressed through the above mechanisms, the Regulator's focus is on assessing risks posed to people other than those participating in the clinical trial, and to the environment.

10. The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use *Guidelines for Good Clinical Practice* (ICH-GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects. The guideline was developed with consideration of the current good clinical practices of the European Union, Japan, and the United States, as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). TGA has adopted the ICH-GCP in principle as *Note for Guidance on Good Clinical Practice* (designated CPMP/ICH/135/95), which provides overarching guidance for conducting clinical trials in Australia which come under TGA regulation.

11. The National Health and Medical Research Council (NHMRC) has issued the *National Statement on the Ethical Conduct in Research Involving Humans* (National Health and Medical Research Council 2013). This document sets the Australian standard against which all research involving humans is reviewed. The *Therapeutic Goods Act 1989* requires that the use of a therapeutic good in a clinical trial must be in accordance with the ethical standards set out in this document.

12. Approval by a HREC is also a fundamental requirement of a clinical trial. HRECs conduct both ethical and scientific assessment of the proposal and in addition often consider issues of research governance. Other elements of governance of clinical trials that are considered by HRECs include appropriate informed consent, specific inclusion and exclusion criteria, data monitoring and vaccine accounting and reconciliation.

Section 4 The Proposed dealings

13. PaxVax has proposed a clinical trial of a genetically modified (GM) live bacterial vaccine against Cholera (named PXVX0200) under limited and controlled conditions in Australia.

14. The dealings involved in the proposed clinical trial will include:

- importing the GMOs;
- conducting experiments with the GMO;
- transporting the GMO;
- disposing of the GMOs; and
- possession, supply or use of the GMOs for any of the purposes above.

15. These proposed dealings are detailed further below.

16. The purpose of the trial is to verify the effectiveness of the vaccine in producing an immune response against cholera.

17. This vaccine is being developed for use as a travel vaccine, for the active immunization against disease caused by *V. cholerae* serogroup O1 in adults and children aged 2 years or older who would be visiting cholera endemic or epidemic areas. It may also be useful for use in developing countries in response to cholera outbreaks.

18. The trial will involve up to 1000 volunteers (children and adults) in Australia. Trial participants will be given 100 ml of GM vaccine suspension as a drink for oral ingestion. This trial would form part of a larger international study including trials in the USA and Canada.

19. Clinical trial of the GM vaccine in Australia would contribute to the following three international clinical studies:

- A Phase III Randomized, Double-blind, Placebo-Controlled Three-Lot Consistency Study in Healthy Adult Volunteers to Assess Immunogenicity, and Clinical Acceptability of a Single-dose of the Live Oral Cholera Vaccine Candidate PXVX0200, *Vibrio cholerae* O1 Serotype Inaba Vaccine Strain CVD 103-HgR.
- A Phase III Randomized, Double-blind, Placebo-controlled Study in Older Adults to Assess Immunogenicity and Clinical Acceptability of a Single-dose of the Live Oral Cholera Vaccine Candidate PXVX0200 *Vibrio cholerae* O1 Serotype Inaba Vaccine Strain CVD 103-HgR.
- A Phase III Randomized, Double-blind, Placebo-controlled Study in Children to Assess Safety and Immunogenicity of the Live Oral Cholera Vaccine Candidate PXVX0200 *Vibrio cholerae* O1 Serotype Inaba Vaccine Strain CVD 103-HgR.

20. The GM vaccine, PXVX0200, will be imported from the USA via courier under temperature-controlled conditions and transported directly to the clinical trial sites.

21. The GM vaccine will be transported under refrigerated conditions and stored in a -20°C freezer as it is susceptible to higher temperatures and it loses its potency if left at room temperature.

22. The GM vaccine will be provided as a lyophilized powder in single-use sachets (8×10^8 CFU), each contained in a 60 x 90 mm multi-layered sealed aluminium pouch.

23. Along with the vaccine there will be a bicarbonate buffer (dry powder) in a similar but separate pouch. The vaccine and buffer sachets will be packed in cardboard cartons and the cartons will be labelled.

24. The preparation of the vaccine will vary depending on whether it is for children or adults. For adults the vaccine will be prepared by adding the contents of a vaccine sachet plus the contents of a buffer sachet into an opaque cup containing 100 mL of water.

25. For children aged between two and six the buffer sachet will be dissolved in 200 mL of water, then 100 mL of this buffer mixture will be discarded, prior to adding the contents of the vaccine sachet. This will halve the concentration of buffer administered to these children to improve the palatability of the vaccine. The reduction in buffer concentration will not adversely affect the vaccine potency, as the total output of acid in the smaller stomach of children is less than that of adults and therefore less buffer is required.

26. Each trial participant will be given the resultant mixture of vaccine (100 ml) as a drink (single dose) and asked to drink the entire amount at the clinical trial site. The trial participant will then be monitored for at least an hour for any evidence of adverse effects and in case the vaccine is regurgitated.

27. The vaccination procedures will be conducted by trained staff and be undertaken at clinical facilities. A list of potential sites has been provided in (Table 1, Chapter 1, Section 4.1).

28. The clinical trial will be conducted in accordance with *Standard Universal Precautions* as established by WHO (WHO-GCP 2014) and ICH-GCP[2] guidelines.

29. Volunteers (and the parents/caregivers of child participants) for the clinical trial will be provided with information regarding the vaccine, the clinical trial and the general hygiene they will be expected to practice during the trial period and will be required to sign an informed consent document.

30. The clinical trial will be divided into three phases:

- Screening: Past medical history of the volunteers will be screened and their suitability to participate in the trial will be assessed according to the exclusion criteria described in Chapter 1, Section 4.2;
- Vaccination: Medical fitness examinations and blood tests of trial participants will be conducted. The liquid vaccine will be given to the trial participant to drink and they will be monitored for 60 minutes in the clinic for any side effects. After the vaccination, the trial participants will be provided a form to be filled out to record how they are feeling after taking the vaccine;
- Post-vaccination: After vaccination, the trial participant will be expected to return to the clinical facility for follow up visits at 11, 29 and 181 days post-vaccination. During the follow up visits, trial participants will provide the form in which they have recorded their post-vaccination experience, blood samples will be taken and an interview conducted to prepare individual participants record.

31. Blood samples will be frozen on site and shipped to an appropriate laboratory for immunological assessments. This would include serum cholera-specific antibody, cholera-toxin IgG antibody, and memory B cells analysis.

[2] The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, *Guidelines for good clinical practice* (ICH 1996) as adopted by the TGA.

32. Some details of the application including the molecular characterisation and batch testing methods for the GM vaccine have been declared Confidential Commercial Information (CCI) under section 185 of the Act. This information was evaluated as part of a separate application for declaration of CCI. Information covered by the CCI application, which was relevant for the risk assessment purposes, was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities consulted on this application.

4.1 The proposed limits of the dealings (scope, scale, locations, duration and people)

33. The trial may take place at nine clinical facilities located in the local government areas listed in Table 1 below, from the date of issue of the licence until after the required number of participants have been enrolled and vaccinated (approximately one year). The applicant intends to enrol a maximum of 1000 volunteer trial participants in Australia. However, if the required number of participants could not be enrolled within one year. The applicant could apply for a variation to the licence and request an extension of period of the licence.

Table 1 Sites where clinical trial with GM vaccine may be conducted

Clinical Facility	Local Government Area	Locality
AusTrials Pty Ltd	City of Brisbane	Sherwood, QLD
QPharm Pty Ltd	City of Brisbane	Herston ,QLD
Cmax Australia Ltd	City of Adelaide	Adelaide, SA
Emeritus Research Pty Ltd	City of Stonnington	Malvern East, VIC
Nucleus Network	City of Banyule	Heidelberg, VIC
Nucleus Network	City of Melbourne	Melbourne, VIC
Royal Children's Hospital	City of Melbourne	Parkville, VIC
The University of Melbourne	City of Melbourne	Carlton, VIC
Linear Clinical Research Ltd	City of Nedlands	Nedlands, WA

4.2 The proposed controls to restrict the spread and persistence of the GM vaccine and its genetic material in the environment

34. The applicant has proposed a number of controls to restrict the spread and persistence of the GM vaccine and to restrict exposure to the GM vaccine including:

- inoculating a maximum of 1000 individuals in Australia with the GM vaccine via oral administration;
- requiring that vaccine administration be performed by trained nurses and/or physicians at health care facilities in accordance with WHO *Standard Universal Precautions* and ICH-GCP;
- storage and transport of all GM vaccine, including any waste or samples containing the GM vaccine, in accordance with relevant guidelines and regulations[3];
- monitoring vaccinated individuals for at least one hour after ingestion to ensure the vaccine solution is not regurgitated;

[3] The Regulator's *Guidelines for the Transport, Storage and Disposal of Genetically Modified Organisms*, IATA Transportation Regulations

- disposing of all waste in accordance with standard clinical waste disposal practices as required by the relevant local and state legislation;
- excluding people who:
  - have travelled to a cholera endemic area in the previous 5 years; or
  - have abnormal stool patterns or regular use of laxatives; or
  - are allergic to tetracycline and/or ciprofloxacin; or
  - have history of cholera or enterotoxigenic *E. coli* challenge; or
  - are pregnant or nursing women; or
  - are children less than two years of age; or
  - are immunodeficient or immunosuppressed (by disease or therapy), including those with HIV infection.
- destroying left over liquid vaccine by chemical methods such as 10% bleach or 70% isopropyl alcohol before disposal at the clinical site following institutional procedures for the disposal of biohazardous material;
- discarding waste generated during the conduct of the study, such as disposable cups and empty vaccine sachets, into appropriate biohazard containers and disposing of the waste at the clinical site following institutional procedures for the disposal of biohazardous material;
- destroying unused study vaccine at the clinical site following institutional procedures for the disposal of biohazardous material such as incineration;
- instructing trial participants to:
  - wash hands in soap and water after using the bathroom and always before handling food or eating;
  - keep hands and any unclean items, or items used for toilet purposes, away from the mouth, eyes, ears, nose, and wounds;
  - avoid use of shared or unclean eating utensils, drinking cups, towels and handkerchiefs; and
- instructing parents/caregivers of child participants to use disposable nappies where necessary, and enclose the used nappies in two sealed plastic bags prior to disposing in the trash, for the 3-4 week period post-vaccination. These instructions will be included within the informed consent document for the paediatric study.

35. Overarching documents such as the Phase III study protocols and pharmacy manual detail procedures and practices, inclusion and exclusion criteria, informed consent, monitoring, auditing, reporting and recordkeeping and other governance and administrative requirements for the study. The Principal Investigator and clinical staff at each site will be responsible for recording clinical information regarding the trial, including the location and date of GM vaccine administration.

36. If a spill of the GM vaccine occurred during preparation, the area will be carefully cleaned using a dilute bleach solution (1 part bleach to 10 parts water) or according to local biohazard requirements. Antibiotic therapy could be provided to exposed personnel, if clinically indicated.

37. In case of accidental exposure to the vaccine, staff will be instructed to perform the following:

- for eye exposure - flush eye continuously with water for at least 15 minutes and obtain immediate medical assistance;
- for skin contact or open wound exposure - remove contaminated clothing and flush exposed area with large amounts of water and soap. Obtain medical assistance if skin reaction occurs such as redness, itching, or swelling; and
- for inhalation exposure - seek medical assistance in case of known or possible over exposure to this material.

38. Written informed consent from each trial participant (or parent/guardian for child participants) will be required for participation in the trial. This will be monitored by the relevant HREC.

39. The study will be monitored on a regular basis throughout the study period by a Safety Monitoring Committee appointed by PaxVax. This committee will monitor compliance with procedures and record keeping, the study protocol, handling of the vaccine and clinical samples, collection of informed consent, and safety reporting according to the HREC requirements for each individual clinical site.

40. These controls and the limits outlined above have been taken into account in establishing the risk assessment context (this chapter), and their suitability for containing the proposed release is evaluated in Chapter 2.

Section 5 The parent organism

41. Cholera is a rapidly dehydrating diarrhoeal disease caused by ingestion of toxigenic serogroups of *Vibrio cholerae* (serogroup O1 and less commonly O139). Cholera is also known as a disease of poverty as it is closely linked to poor sanitation and a lack of clean drinking water (WHO 2014).

42. Since 1817, seven cholera pandemics have spread from Asia to much of the world. The seventh pandemic, which is still in effect, began in Indonesia in 1961 and spread through Asia to Africa, Europe and Latin America. The WHO estimates that 3–5 million cases of cholera occur per year, killing 120 000 people annually. Outbreaks occur predominantly in Asia and Africa, with periodic major epidemics elsewhere, including the Haiti epidemic that began in 2010 (Harris et al. 2012).

43. The 2010 cholera epidemic in Haiti was determined to be caused by contamination of a local river with a pathogenic strain of wild-type *V. cholerae* serogroup 01 which was identical to a strain circulating in Nepal at that time. The contamination was determined to be as a result of human activity following the earthquake (Lantagne et al. 2013).

44. The provision of safe drinking water and adequate sanitation, along with health education, food safety measures, and strong disease surveillance remain the mainstays of preventing both endemic and epidemic cholera (WHO 2013).

45. The parent organism from which the GM vaccine was derived is *V. cholerae* strain 569B, which was first isolated from a patient in 1948 in India (Bik et al. 1996). In comparison to other pathogenic *V. cholerae* strains, *V. cholerae* strain 569B is considered to be a weak pathogen as it does not produce two of the toxins associated with severe cholera disease: haemolysin (a toxin that breaks down the red blood cells) and shiga-like toxin (also involved in causing diarrhoea). However, it is a pathogenic strain and it does produce cholera toxin which causes cholera disease (Alm et al. 1988).

5.1 Basic biology

46. *V. cholerae* is a facultative anaerobic, gram negative rod shaped bacteria. Facultative anaerobes can survive both in aerobic conditions (in the presence of oxygen) as well as anaerobic conditions (in the absence of oxygen) such as inside a human gastrointestinal tract.

47. Different strains of *V. cholerae* are distinguished into serogroups on the basis of O antigens on the cell surface. The *V. cholerae* predominantly associated with epidemic cholera is *V. cholerae* serogroup O1 (Kaper et al. 1995).

48. The *V. cholerae* genome consists of two circular chromosomes. Both chromosomes are indispensable as they each carry essential genes. Most of the genes required for growth and viability are located on the larger chromosome (chromosome one), although some genes essential for normal cell function are found only on chromosome two (Heidelberg et al. 2000; Trucksis et al. 1998).

49. *V. cholerae* is a conjugative bacterium; that is, it is known to exchange genetic elements with other compatible bacteria present in the surrounding environment. Genetic elements are thought to move horizontally (to bacteria of the same or different species) and vertically (to offspring) as they can help bacteria adapt to changing environments. In order to exchange mobile genetic material, two compatible bacteria form a conjugation/mating tube which joins the two bacteria. Mobile genetic elements and plasmids are then transferred via the tube (Kaper et al. 1995).

50. Individual *V. cholerae* may also contain a variety of plasmids and other mobile genetic elements encoding genes specific to the environment in which they are found. Comparative genomic studies have also identified a large 'mobilome' composed of mobile genomic islands in the *V. cholerae* genome (Cho et al. 2010).Chromosomal genes are indispensable for the bacteria and these genes are usually very stable and not easily transferred. However, some *V. cholerae* strains carry a large P plasmid (68 kb in size), also known as fertility factor, which mediates chromosomal gene transfer via conjugation (Bhaskaran 1959). P plasmids are not very common in *V. cholerae* strains and they were not detected in three separate studies in which more than 266 *V. cholerae* strains were examined (Kaper et al. 1994). Additionally, strains possessing this plasmid were found to be avirulent in animal models (Bartowsky et al. 1990; Sinha & Srivastava 1978). Other than P plasmid, different *V. Cholerae* strains carry a few smaller plasmids.

51. *V. Cholerae* strain 569B does not carry P plasmid, it only has one small plasmid (4.5-5 kb) and no specific function could be attributed to the smaller plasmid (Viret et al. 2004). When the P plasmid was experimentally introduced into *V. cholerae* strain 569B, although cholera toxin production was not affected, the ability of the strain to colonise the intestine was reduced five-fold in the rabbit illeal loop model (Bartowsky et al. 1990). More recently, another large (80 kb) conjugative plasmid (p3iANG) was identified in some clinical and environmental strains of *V. cholerae* isolated from Africa. This plasmid carries several mobile genetic elements also known as Integrative and Conjugative Elements (ICEs). Some of the ICEs harbour multiple antibiotic resistance genes and are able to mobilise plasmids and chromosomal DNA from strain to strain (Ceccarelli et al. 2006; Valia et al. 2013).

52. Bacteriophage mediated transfer of mobile genetic elements is also common in bacterial populations (Parker & Romig 1972). A bacteriophage is a type of virus that infects and replicates in susceptible bacteria. Bacteriophages can integrate their genome into bacterial genomes, replicate inside a bacterial cell and produce progeny bacteriophage that can carry mobile genetic material from one bacterium to another.

53. In some strains of *V. cholerae*, part of the genome that encodes the cholera toxin (*ctxAB*) genes also includes sequences that encode a transmissible bacteriophage (prophage sequences), which, if activated, can produce infectious bacteriophage. This bacteriophage is able to transfer

the *ctxAB* genes from *ctxAB* positive donor strains to *ctxAB* negative recipient strains in the mouse gastrointestinal tract. The bacteriophage gains entry to the bacterial cell via toxin co-regulated pili (TCP) (Mekalanos et al. 1997; Waldor & Mekalanos 1996a).

54. The parent organism used to generate the GM vaccine strain is a non-permissive host for this bacteriophage, making it immune to the bacteriophage infection. Additionally, if the endogenous prophage sequences are activated, it produces only defective bacteriophages that are unable to infect other V. cholerae strains (Viret et al. 2004).

5.2 Basic environmental biology

55. *V. cholerae* is found in the normal, free living bacterial flora in estuarine environment (where fresh water from rivers and streams mixes with salty ocean water) and coastal waters particularly in tropical and subtropical regions. These organisms grow best in warm water that contains sufficient organic nutrients.

56. *V. cholerae* can persist in environmental waters in a spore-like dormant state that is not culturable but is infectious. Such a dormant state has been described for a number of bacterial species as a survival strategy in the natural environment. In this dormant state, the cells are reduced and ovoid (Kaper et al. 1995). *V. cholerae* is often associated with zooplankton and chitinous shelled animals, and vegetation. However, these are only carriers rather than hosts (i.e. they are not infected by the bacteria) (Reidl & Klose 2002).

57. Copepods, small aquatic animals with a chitin shell, have been identified as a reservoir for both culturable and non-culturable *V. cholerae* (Colwell 2002). The bacteria can survive for extended periods in the copepod intestines or attached to copepod chitin shells (Rebaudet et al. 2013).

58. Cholera is endemic in southern Asia and parts of Africa and Latin America, where seasonal outbreaks occur widely and are particularly associated with poverty and poor sanitation. However, *V. cholerae* also persists in the Australian environment and has been isolated from river systems in the east and north-west of Australia, including the Rockhampton, Brisbane, Lismore, and Sydney areas (Desmarchelier et al. 1995a).

59. Although both pathogenic and non-pathogenic forms of *V. cholerae* persist in the Australian environment, cholera infections are rare, due to stringent domestic water treatment, waste disposal and sewage treatment measures.

5.3 The cholera toxin and disease

60. *V. cholerae strain 569B* contains two copies of the genes encoding the cholera toxin (*ctxAB),*with one copy located on each of the two chromosomes. The Cholera toxin (Ctx) is a well characterised hexameric protein consisting of one A-subunit arranged on a ring of 5 B-subunits. The A subunit is composed of the toxic and enzymatically active A1 fragment and the helically structured A2 fragment which forms a link to the B subunit pentamer (Figure 2).

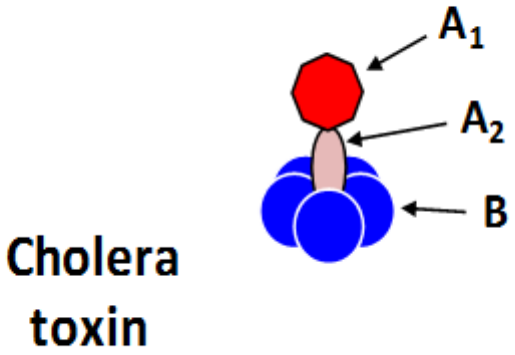

Figure 2. Structure of cholera toxin

61. Only the A1 fragment has toxin activity. The A2 fragment and B subunits are non-toxic, however they can elicit a cholera toxin-specific immune response. The B subunit mediates specific binding to the ganglioside receptors present on all nucleated cells including epithelial cells of the small intestine (Spangler 1992). Once bound to the human epithelial cell, the A2 and B subunits help the A1 subunit to enter the cell.

62. Once inside the cell, the A1 fragment binds to proteins which are involved in transmitting signals / stimuli to the cells (G proteins). This induces a cascade of events in the epithelial cells, such as a decrease in the net flow of sodium into intestinal epithelial cells, which in turn produces a net flow of water and chloride ions out of the cells. These events lead to the dramatic loss of water characteristic of *V. cholerae* infection (Mekalanos et al. 1997; Spangler 1992).

63. Although cholera toxin is responsible for the profuse diarrhoea typical of cholera, there are other proteins expressed by *V. cholerae* O1 which play a role in the virulence of the bacterium. Some of the well characterised proteins are: Zot, which can increase the permeability of the small intestinal mucosa; Ace, which can cause fluid accumulation; Haemolysin/cytolysin, which can lyse (break open) a variety of erythrocytes and mammalian cells in culture, and Shiga-like toxin, which is known to cause similar effects as cholera toxin (Kaper et al. 1995).

64. *V. cholerae* strain 569B (the parent strain of PXVX0200) has reduced pathogenicity in comparison to other pathogenic strains of *V. cholerae* serogroup O1, which is predominantly associated with epidemic cholera. Although *V. cholerae* strain 569B produces the cholera toxin, it does not produce a Shiga-like toxin which also causes diarrhoea. It also is unable to produce a functional haemolysin due to a naturally occurring 11 base pair deletion in the haemolysin (*hlyA*) gene. This results in a truncated, non-functional haemolysin protein which is not able to break down red blood cells (Alm et al. 1988).

65. *V. cholerae* is sensitive to the low pH found in the human stomach and, for the onset of severe cholera in an otherwise healthy person, a high infectious dose of more than a million ($\sim 10^6$ to $\sim 10^8$) live bacterial particles is required. However, the infectious dose can drop to $\sim 10^4$ bacteria in individuals who produce less stomach acid, including young children, the elderly and those who take antacids (Kitaoka et al. 2011).

66. *V. cholerae* colonises but does not penetrate the intestinal mucosa. The bacteria pass through the human gastric acid barrier into the small intestine where they colonise, multiply and begin to secrete cholera toxin (Kaper et al. 1995).

67. During the acute phase of cholera up to half the body weight can be lost in 24 hours (Wilson 1984). If untreated, this massive loss of water leads to dehydration and the rapid collapse of the circulatory system, which is the main cause of death among cholera patients. Mortality in untreated patients is greater than 60% and, due to the massive loss of water and electrolytes, death occurs as early as 18 hours. Antibiotic treatment is of limited efficacy because of the rapid onset of symptoms. However, if patients are sufficiently rehydrated during the acute phase of the disease, survival rate is nearly 100%.

5.4 Host range and transmissibility

68. The only natural host for *V. cholerae* are humans and there have been no reports of the bacteria causing disease in other animals even in areas where cholera is endemic. Cholera is typically spread by consumption of water that is contaminated with human faeces. However, it can also be transmitted by contaminated seafood, which can acquire the organism from environmental sources. Direct transmission from person to person is uncommon (Kaper et al. 1995; WHO 2014).

69. Pathogenic *V. cholerae* have filaments attached to the bacterial cell called Toxin Co-regulated Pili (TCP). TCPs are found only in pathogenic strains as they allow the bacteria to attach to and colonise cells in the human intestinal mucosa. TCPs are very specific to the cell type they can recognise. *V. cholerae* TCPs can only recognise human intestinal cells. Therefore, they are also known as adhesion factor or colonisation factor and play a major role in restricting the host range of pathogenic *V. cholerae* to humans (Kaper et al. 1995).

70. Aerosol transmission is not reported in the available literature possibly due to the high infectious dose of more than a million ($\sim 10^6$ to $\sim 10^8$) live bacterial particles that are required to establish the infection and cause disease.

71. The incubation period for cholera can range from a few hours to five days after infection. Symptomatic patients may shed bacteria before developing the clinical signs of illness, and for up to two weeks after infection, whereas asymptomatic patients typically only shed bacteria for a day (Harris et al. 2012; Reidl & Klose 2002).

5.5 Susceptibility to antibiotics and other chemical agents

72. *V. cholerae* strains remain susceptible to commonly used antibiotics. However, strains resistant to one or more antibiotics have also been reported. *V. cholerae* are susceptible to commonly used disinfectants such as 1% sodium hypochlorite, 4% formaldehyde, 2% glutaraldehyde, 70% ethanol, 70% propanol, 2% peracetic acid, 3-6% hydrogen peroxide, and 0.16% iodine (Centers for Disease Control and Prevention 2013).

Section 6 The GM vaccine, nature and effect of the genetic modification

6.1 Introduction to the GM vaccine

73. The GM vaccine contains the genetically modified bacterium *V. cholerae* strain CVD 103-HgR which is not able to cause disease (Ketley et al. 1993a). The parent strain, *V. cholerae*

strain 569B, from which the vaccine strain was derived, contains two copies of the cholera toxin genes (*ctxAB*), one on each chromosome, and a single non-functional copy of the haemolysin (*hlyA)* gene on its smaller chromosome. The vaccine strain has been produced by deleting 94% of both chromosomal copies of the cholera toxin A subunit (*ctxA*) gene and inserting the mercury resistance (*mer*) genes from *Shigella flexneri* into the *hlyA* gene as a marker (Figure 3).

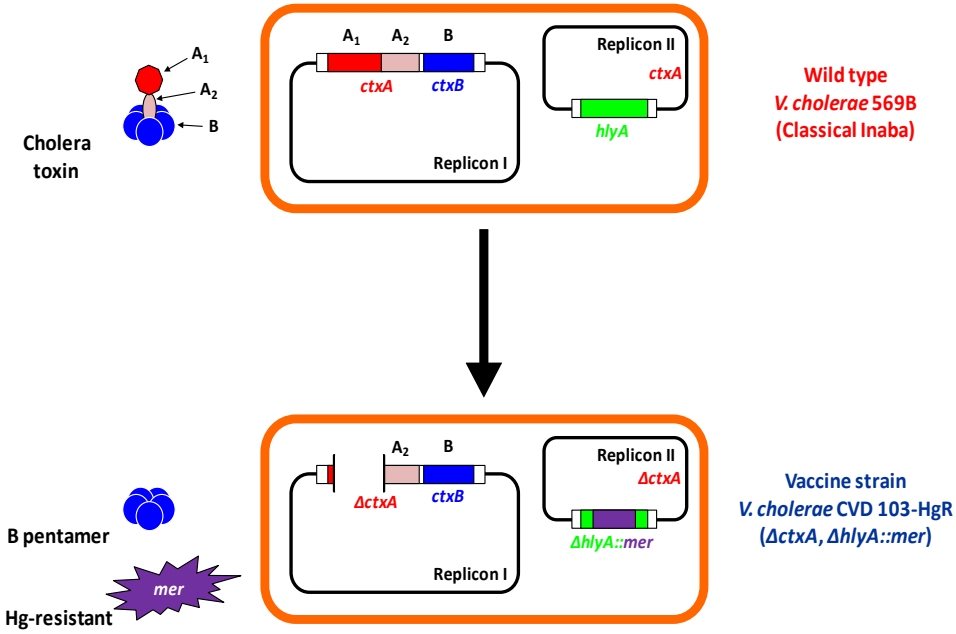

Figure 3. Construction of the GM vaccine

74. The two cholera toxin B subunit (*ctxB*) genes remain intact in the vaccine strain. The cholera toxin B subunit, however, is not toxic or able to cause disease in the absence of the A subunit. Vaccination with the genetically modified bacteria has been shown to induce an immune response that protects against infection with cholera toxin-producing O1 serogroup *V. cholerae* (Chapter 1, Section 6.4.4).

6.2 Method of the genetic modification

75. The GM vaccine was generated through a series of complex cloning steps. The process can be largely divided into two parts, deletion of the majority of the *ctxA* gene; and insertion of a mercury resistance operon.

6.2.1 ctxA gene deletion

76. The GM vaccine has had a 550 base pair fragment of DNA encoding the A1 peptide of cholera toxin removed. However, as there is no easy way to select for the loss of *ctxA* gene expression, an antibiotic resistance trait was introduced in an intermediate step.

77. First, a chromosomal DNA fragment from *V. cholerae* strain 395 containing the *ctx* operon (encoding the *ctxA1*, *ctxA2* and *ctxB* genes) was inserted into a cloning plasmid (Kaper et al. 1984). A tetracycline resistance gene (*tet*) was then inserted into the coding sequence for *ctxA1*, preventing *ctxA1* expression.

78. The modified *ctx* operon (*ctxA::tet*) was excised using restriction enzymes and was inserted into a broad host range mobile bacterial plasmid. This mobile plasmid was then transferred into the *V. cholerae* 569B strain via bacterial conjugation. Homologous recombination between the host chromosomes and the plasmid resulted in swapping of the modified genes from the plasmid to each of the bacterial cell chromosomes in place of the endogenous *ctx* genes. The antibiotic tetracycline was then used to select for *V. cholerae* in

which both chromosomal copies of the *ctx* operon had been replaced with the genetically modified *ctxA::tet* operon. The resultant strain was named *V. cholerae* JBK126.

79. Second, in a separate procedure, site specific restriction enzymes were used to remove a 550 base pair fragment of DNA encoding the A1 peptide of cholera toxin from the *ctx* operon that was cloned as described in paragraph 77.

80. The modified *ctx* operon (Δ *ctxA*) was excised using restriction enzymes and was inserted into a broad host range mobile bacterial plasmid, and this plasmid was transferred into *V. cholerae* JBK126. As above, homologous recombination resulted in the Δ *ctxA* operon from the plasmid replacing the ctxA::tet operon in each of the bacterial chromosomes. *V. cholerae* that were no longer resistant to tetracycline, in which both the genomic copies of the *ctxA::tet* operon were replaced with the deleted *ctx* operon (Δ *ctxA*), were selected. The resultant strain was named *V. cholerae* CVD 103 (Kaper et al. 1984).

6.2.2 mer operon insertion

81. Insertion of the mercury resistance operon into *V. cholerae* CVD 103 was carried out via a similar process as used for the *ctxA* gene deletion. A chromosomal DNA fragment from *V. cholerae* N16961 containing the haemolysin (*hlyA*) gene was inserted into a cloning plasmid. A 400 base pair fragment of DNA was deleted from the cloned *hlyA* gene and replaced with a 4.2 kb DNA fragment containing the *S. flexneri mer* operon (Ketley et al. 1993b).

82. The modified *haemolysin* gene (Δ *hlyA::mer*) containing the *mer* operon was transferred to the *V. cholerae* CVD 103 strain using the same process of homologous recombination involving a *hlyA::tet* intermediate, as described in Section 6.2.1 above.

83. The resultant strain was named *V. cholerae* CVD 103-HgR, which is the strain used in the GM vaccine.

6.3 The introduced genes, encoded proteins and their associated effects

6.3.1 Genetically modified ctxA *gene*

84. The *ctxA* and *ctxB* genes are arranged on a single transcription unit (operon), within which the *ctxA* gene precedes the *ctxB* gene (Mekalanos et al. 1983). The cholera toxin gene used for generating the GM vaccine was originally isolated from the *V. cholerae* strain 395 (Classical Ogawa). This cholera toxin gene was modified to delete 550 base pairs of DNA and subsequently the modified cholera toxin gene was inserted into *V. cholerae* 569B as described above (Chapter 1, Section 6.2).

85. Due to inactivation of both copies of the *ctxA* gene, the resultant GM *V. cholerae* strain is not able to produce the A1 subunit of cholera toxin and therefore loses its toxic effect (Kaper et al. 1994). As the *ctxB* gene remains intact, the GM *V. cholerae* strain can produce the immunogenic B subunit of the cholera toxin (see Figure 3).

6.3.2 Genetically modified hlyA *gene carrying the* S. flexneri *mer operon*

86. The *hlyA* gene used for generating the GM *V. cholerae* strain was originally isolated from the *V. cholerae* strain N16961. A 400 base pair fragment of DNA was deleted from this *hlyA* gene and replaced with the mercury resistance (*mer*) operon of *S. flexneri*.

87. The GM *hlyA* gene carrying the *mer* operon was introduced into the genome of *V. cholerae* strain 569B via homologous recombination. Therefore, the GM vaccine is not able to produce functional haemolysin.

88. The *S. flexneri mer* operon was inserted into the *hlyA* gene to allow easy and rapid differentiation between the GM *V. cholerae* strain and the wild-type toxin-producing *V. cholerae* (Ketley et al. 1993b).

89. *Shigella spp*. are bacteria that cause shigellosis, also known as bacillary dysentery. The *mer* operon is naturally carried by *S. flexneri* within transposon Tn*21* on a plasmid NR1 (Liebert et al. 1999a). *S. flexneri* is a conjugative bacterium and the *mer* operon is widely distributed among the bacterial community particularly within bacterial species growing in environmental waters contaminated with mercury (Boyd & Barkay 2012; Moller et al. 2014; Sone et al. 2013).

90. A wide range of bacteria like Shigella carry the *mer* operon which helps them survive in mercury contaminated environment by resisting and subsequently transforming the toxic forms of mercury to nontoxic forms. The *mer* operon is naturally spread between bacterial species (Bogdanova et al. 1998). In most Gram-negative bacteria, the *mer* operon is carried on mobile genetic elements, such as transposons and plasmids, facilitating horizontal gene transfer (Brown et al. 2002).

91. The *S. flexneri mer* operon contains two regulatory genes (*merR* and *merD*), four mercury transport genes (*merT, merP, merC* and *merE*), and the mercuric reductase gene (*merA*) (Brown et al. 2002). The distribution of individual mercury operon genes among different bacterial species is variable (Boyd & Barkay 2012).

92. The MerR regulatory protein undergoes an allosteric change when it binds divalent mercury that allows the *mer* operon to be transcribed. When mercury is no longer present the merD protein down-regulates *mer* gene transcription.

93. MerP is a periplasmic protein and binds the divalent mercury ions present in the space between the outer and inner membranes of Gram negative bacteria, such as *V. cholerae*. MerT, MerE and MerC are inner membrane transport proteins. It has been proposed that MerP transfers the mercury ion to the inner membrane transport proteins, which transport the mercury ions to the cytoplasmic mercury reductase, MerA, which breaks down toxic mercury ion into harmless elemental mercury which then evaporates from the bacterial cell surface (Boyd & Barkay 2012; Sone et al. 2013). This helps the bacteria to survive in waters contaminated with mercury.

94. The *hlyA* gene is not associated with the host range determinants of *V. cholerae* (Stonehouse et al. 2008). Insertion of *mer* operon into the *hlyA* gene is not likely to affect the host range of the GM *V. cholerae* strain, which would remain restricted to humans.

6.3.3 Toxicity associated with the genetic modifications

95. As discussed above, the role of the *mer* operon is to protect bacteria from mercury that may be present in the environment. The *mer* operon does not encode genes that can produce, store or sequester mercury. Therefore, there is no mercury toxicity associated with the vaccine due to the genetic modification.

96. Similarly, loss of cholera toxin expression and loss of haemolysin expression in the GM vaccine strain does not change the expression of other genes or result in the production of any novel toxic proteins or other toxic substances.

97. Proteins encoded by the *mer* operon are discussed above in Chapter 1, Section 6.3.2. The DNA and protein sequences of the *mer* operon are known. None of these proteins are known to have a toxic effect, have the ability to cause a significant adverse reaction, or to contribute to the disease caused by *V. cholerae* (Barrineau et al. 1984).

98. Certain members of the human gut microbiota such as lactobacilli naturally carry the *mer* operon (Monachese et al. 2012; Osborn et al. 1997). Therefore, under certain conditions (see below), proteins expressed by the *mer* operon can be expressed inside the human gastro intestinal tract by bacterial species naturally colonising the human gut.

99. The *mer* operon is an inducible operon and it is only activated in the presence of mercury ions. However, a low level of merR expression may occur even in the absence of mercury. In

laboratory conditions, the *mer* operon is activated when it comes in contact with over 20 µM of mercuric chloride (Viret *et al* 2004). Therefore, proteins encoded by *mer* operon, other than merR, are not usually produced inside a human host unless that person consumes mercury or food contaminated with mercury (for example seafood).

100. Similarly, outside a human host, the *mer* operon is likely to be expressed only when the host bacteria are in water contaminated with over 20 µM of mercuric chloride. However, the mercuric ion concentrations in polluted waters typically range from 1 to 10 nM, which is substantially less than the 20 µM required for the activation of *mer* operon (Viret *et al* 2004).

101. Safety of the GM vaccine has been extensively tested in several human clinical trials. No significant toxicity or adverse events which can be attributed to the proteins of the *mer* operon have been reported in any of these studies (See Chapter 1, Section 6.4.4 for details of these studies).

6.4 Characterisation of the GM vaccine strain, *V. cholerae* CVD 103-HgR

102. The GM cholera vaccine strain was tested for antibiotic sensitivity and was found to be sensitive to a range of antibiotics including chloramphenicol, trimethoprim/sulfamethoxazole, tetracycline, ampicillin, norfloxacin, nitrofurantoin, erythromycin and doxycycline (Kaper et al. 1984).

6.4.1 Genetic stability and molecular characterisation

103. The genome of the GM cholera vaccine strain has been fully sequenced and, other than the *mer* operon, there are no additional genomic sequences (e.g. antibiotic resistance genes) present in the genome. Sequence details of the vaccine strain have been declared as Confidential Commercial Information (CCI) under section 185 of the Act. This information was evaluated as part of a separate application for declaration of CCI. Information covered by the CCI application, which was relevant for the risk assessment purposes, was considered during the preparation of the RARMP and was made available to the prescribed expert groups and authorities consulted on this application.

104. The genetic stability of the GM cholera vaccine was studied after prolonged storage in a lyophilized state and outgrowth for 16-17 generations. No differences were detected using a wide variety of restriction enzymes and probes (Favre et al. 1996).

105. Complete genetic stability was observed following immunization and passage through the gut for isolates from the stools of 11 trial participants selected from three independent clinical trials (Favre et al. 1996). Therefore, it was concluded that no genetic rearrangements occurred during transit of the bacteria through the digestive tract either at the *ctxA* deletion site or at the *hlyA* deletion/*mer* insertion site. A probe specific to the *ctxA* gene was unable to bind to DNA from the above isolates, which confirmed that a wild-type *ctxA* gene had not been reacquired by any of the tested isolates (Favre et al. 1996).

106. PaxVax recently conducted further assessment of stability and homogeneity by PCR analysis of 16 individual colonies, generated from the expanded progenitor strain. All 16 colonies were positive for the *mer* operon, an indication of the expected high stability of the chromosomally integrated *mer* operon (information provided in DIR 126 Application).

6.4.2 Studies on shedding and persistence of the GMO into the environment

107. Toxigenic *V. cholerae* are excreted by untreated patients for up to two to three weeks post infection, but rarely for longer periods (Dizon et al. 1967; Kaper et al. 1984). Data from clinical trials have demonstrated that only 30% or fewer of GM vaccine recipients excrete detectable numbers of the GM bacteria for a maximum of 7 days (Kotloff et al. 1992; Lagos et al. 1999; Levine et al. 1988; Simanjuntak et al. 1993).

108. A safety, immunogenicity and transmissibility study of CVD 103-HgR involving 303 children (24 to 59 months-old) and their household contacts (including siblings) was conducted in Indonesia. Overall the vaccine was found to be safe and immunogenic with no significant adverse reactions in children. This study also concluded that transmission rates between the vaccinee and their household contacts were low. The vaccine was minimally excreted and was isolated from only 1 of 177 (0.66%) unvaccinated family contacts (a mother) (Simanjuntak et al. 1993).

109. In a Phase I study conducted by PaxVax, faecal shedding of the GM cholera vaccine was detected in 11% of vaccine recipients within seven days of vaccination. The GM vaccine strain was not detected in either stool samples or rectal swabs collected seven days post-vaccination from available household contacts of vaccine recipients. However, the household contacts tested were adults and did not include children. Therefore, transmission of the vaccine strain from the trial participant to children was not investigated in this study (Chen et al. 2013a).

110. Excretion of GM bacteria from trial participants peaked at 200 bacteria per gram of faeces on day seven, with significant reductions on subsequent days. Therefore, during the peak day of excretion of GM bacteria, less than one third of recipients excreted approximately 4×10^4 live GM bacteria.

111. Moore swabs (4 cm thick gauze rolls attached to a nylon string) were used in an attempt to isolate the GM cholera vaccine strain from toilets and sewers near 97 households of people who had received the vaccine. This study was undertaken in areas where cholera is endemic (Simanjuntak et al. 1993). Samples were taken from where the household effluent entered the sewage drains and from toilets. The vaccine strain was not isolated from any of the samples. Non-O1 *V. cholerae* (i.e. strains other than the vaccine strain) were isolated from 46 of the samples.

112. *V. cholerae* does not survive well in dry conditions such as on fabric, paper, plastic and metal surfaces and dies off within a few days (Felsenfeld 1965; Harris et al. 2012). The GM cholera vaccine is required to be transported in refrigerated conditions and stored in a -20°C freezer because it is susceptible to higher temperatures and loses its potency if left at room temperature for extended periods.

113. Fourteen days after inoculating non-sterile estuarine water with 2.2×10^5 CFU of the GM vaccine no cells could be cultured from the water. Similarly, no GM vaccine could be cultured from soil samples that had been inoculated with approximately 10^6 CFU of the bacteria 19 days previously. Although these studies did not test for the presence of genetically modified bacteria that may have entered the viable but non-culturable state, the data demonstrate that the modified bacteria do not multiply in soil or estuarine water (Viret et al. 2004).

6.4.3 Studies on potential of horizontal gene transfer and potential for reversion of the GM vaccine strain to a pathogenic form

114. Mating experiments using the GM vaccine strain and its parent strain, *V. cholerae* 569B, have been carried out (Kaper et al. 1994). No transfer of the *ctxA* gene to the vaccine strain was detected. Progeny of the vaccine strain were identified using the introduced mercury resistance trait. DNA hybridisation analysis of mercury-resistant colonies was carried out to screen for colonies which had acquired intact *ctxA* genes. No transfer of the *ctxA* gene from the parent strain to the vaccine strain was detected among 120,000 colonies analysed. Similarly, mating experiments between a *V. cholerae* strain harbouring the IncC plasmid and the GM vaccine did not detect gene transfer to the vaccine strain.

115. The potential for transfer of functional *ctxA* gene to *V. cholerae* CVD 103-HgRSR (a spontaneously streptomycin-resistant derivative of the vaccine strain), which would cause the reversion of the attenuated vaccine strain to a pathogenic strain, was also investigated (Kaper et al. 1994). Negative results were obtained in mating experiments using three different strains of

V. cholerae as potential sequence donors, each with an antibiotic marker gene inserted into the *ctxA* gene.

116. Varying the donor/recipient ratios, using broth instead of plate cultures and carrying out the experiments in marine water samples for 11 days did not produce any positive results. The test was also done in the intestines of suckling mice, where no *ctxA* gene transfer to the vaccine strain was detected. Therefore, no evidence of reversion of GM vaccine strain to pathogenic form was observed (Kaper et al. 1994).

117. One gene transfer event of the *ctxA* gene was observed when the GM vaccine was mated with the *V. cholerae* strain JBK56, which contains a P fertility plasmid. In this system, gene transfer frequency was 1.3×10^{-8}. However, P plasmids are very rare in virulent *Vibrio* strains (Kaper et al. 1994).

118. As discussed in Chapter 1, Section 5.1, the GM vaccine strain and its parent (strain 596B), are non-permissive hosts for the most common *V. cholerae* bacteriophage. To confirm this, both the vaccine and the parent strain were cultured along with infectious bacteriophages. Bacteriophages failed to infect or lyse the bacterial cells, confirming that the GM vaccine strain and its parent strain are immune from the bacteriophage infection. Additionally, the prophage sequences present in the genome of the GM vaccine strain are not able to produce any infectious bacteriophage particles (Favre et al. 1996).

6.4.4 Results of previous clinical trials

119. Extensive clinical studies in North America, Europe, South America, Asia and Africa have established the safety and immunogenicity of the GM vaccine. A summary of these is presented in Table 2. Adverse events were typically gastrointestinal in nature, predominantly nausea, diarrhoea, abdominal discomfort, and vomiting, with reports also of headache, rash, and fever. These were mild to moderate and generally consistent with the placebo with respect to frequency and severity.

Table 2 Summary of previous clinical trials

No.	Study Design (Location)	No. Treated	Key Safety findings	Reference
1	Open-label, single-centre, single-dose study (One centre US)	105 Healthy Volunteers (HV) (15 treated)	No significant symptoms were reported	(Levine et al. 1988)
2	Randomized, double-blind, placebo-controlled study investigating safety and immunogenicity. (One centre in Thailand)	24 HV (12 treated / 12 placebo)	No significant differences in adverse events compared with placebo	(Migasena et al. 1989)
3	Randomized, double-blind, placebo-controlled study investigating safety and immunogenicity following a single dose (One centre Switzerland)	50 HV (25 treated / 25 placebo)	Two patients in each group reported diarrhoea which did not interfere with normal activities	(Cryz, Jr. et al. 1990)
4	Randomized, double-blind, placebo-controlled, crossover study investigating safety and immunogenicity following a single dose (One centre US)	94 HV	No adverse events attributable to vaccine	(Kotloff et al. 1992)
5	Open-label, single-centre, single-dose study investigating safety and immunogenicity following a booster dose (One centre Switzerland)	21 HV	One patient reported transient moderate diarrhoea	(Cryz, Jr. et al. 1992)
6	Two open label, single-centre, single-dose studies investigating duration and onset of immunity (One centre US)	33 HV 7 HV	No significant symptoms were reported	(Tacket et al. 1992)

No.	Study Design (Location)	No. Treated	Key Safety findings	Reference
7	Randomized, double-blind, placebo-controlled study investigating safety, immunogenicity and excretion following a single dose (One community, Indonesia)	412 HV paediatrics 5-9 years old (314 treated / 98 placebo)	No significant differences in adverse events compared with placebo	(Suharyono et al. 1992)
8	Four studies investigating safety and immunogenicity: Study 1: randomized to treatment or placebo Study 2: randomized to one of two batches of vaccine Study 3: open-label Study 4: 6 group double-blind cross-over study with patients either receiving 1 or 2 doses of vaccine (with placebo, One centre, Thailand)	429 HV (324 treated / 105 placebo)	No significant differences in adverse events compared with placebo in either of the placebo-controlled studies (1 and 4); no diarrhoeal illness was observed in studies 2 or 3.	(Su-Arehawaratana et al. 1992)
9	Two randomized, double-blind, placebo controlled studies investigating safety, immunogenicity, and transmissibility (Communities in Jakarta, Indonesia)	303 Paediatrics 24-59 month old (155 treated / 148 placebo)	No significant differences in gastrointestinal adverse events compared with placebo. Fevers were significantly higher in the vaccine treatment groups (P=0.045). The fevers were low grade and clustered in the youngest children; with a statistically significant relationship between age and occurrence of fever (P=0.02).	(Simanjuntak et al. 1993)
10	Randomized, double-blind, placebo-controlled study investigating safety, immunogenicity and excretion following a single dose (One community, Peru)	247 HV (163 treated / 84 placebo)	No adverse events attributable to vaccine	(Gotuzzo et al. 1993b)
11	Safety and immunogenicity of a live oral bivalent typhoid fever (*Salmonella typhi* Ty21A)-Cholera (*Vibrio cholerae* CVD 103-HgR) vaccine (One centre Austria)	185 HV (30 monovalent typhoid; 35 monovalent CVD 103-HgR; 60 bivalent therapy; 60 monovalent typhoid followed by bivalent)	144 reactions reported: 131 mild; 13 moderate. None required curtailing normal activities.	(Cryz et al. 1995)
12	Randomized, double-blind, placebo-controlled, crossover study investigating safety and immunogenicity following a single dose (Centres in Sikasso and Bamako, Mali)	38 HIV-seropositive patients and 38 HV	No significant differences in adverse events compared with placebo	(Perry et al. 1998)
13	Randomized, double-blind, placebo-controlled, multi-centred efficacy study in preventing cholera following challenge with *V. cholerae* O1 El Tor Inaba three months after vaccination (Two centres US)	85 HV (43 treated / 42 placebo)	No statistically significant differences in adverse events compared with placebo	(Tacket et al. 1999)

No.	Study Design (Location)	No. Treated	Key Safety findings	Reference
14	Randomized, double-blind, placebo-controlled efficacy study investigating safety and immunogenicity following a single dose (65 communities in Jakarta, Indonesia)	67,508 paediatric and adult subjects (33696 treated / 33812 placebo)	No significant differences in adverse events compared with placebo (1077 patient monitored subgroup)	(Richie et al. 2000)

120. Although not part of a clinical trial, data was also collected when vaccination with the GM vaccine was used as part of control measures to limit a cholera outbreak in Pohnpei Island, Micronesia, in 2000-2001. More than 14 000 people were vaccinated and vaccine efficacy was estimated at 79.2%. This suggests that vaccination with the GM vaccine can be a useful tool for controlling outbreaks (Calain et al. 2004).

121. In industrialised countries and urban areas of developing countries where cholera is not endemic, the GM vaccine displayed significant antibody response in > 90% of healthy volunteers who received a dose of 5×10^8 CFU (Cryz, Jr. et al. 1990; Kotloff et al. 1992; Levine et al. 1988; Migasena et al. 1989; Su-Arehawaratana et al. 1992; Tacket et al. 1992). However, in areas with endemic cholera, the results were variable and 15-75% of subjects displayed significant antibody response (Gotuzzo et al. 1993a; Perry et al. 1998; Richie et al. 2000; Simanjuntak et al. 1993; Su-Arehawaratana et al. 1992; Suharyono et al. 1992).

122. PaxVax recently conducted a Phase I study to evaluate the safety and immunogenicity of the GM cholera vaccine (PXVX0200) prepared from new master and working cell banks in healthy male and female adults. Initial results confirmed that the newly manufactured product has a similar safety and immunogenicity profile as the previously manufactured product (Chen et al. 2013b).

Section 7 The receiving environment

7.1 Relevant environmental factors

123. The primary environment receiving the GM cholera vaccine would be the gastrointestinal (GI) tract of the trial participants. The mucosal surface of the GI tract is colonized by around 400 different species and subspecies of bacteria (Hao & Lee 2004). Human gut microbiota would come into contact with heavy-metals such as mercury and other contaminants when they are ingested through diet.

124. Certain members of the human gut microbiota, such as lactobacilli used in food applications and probiotics, naturally carry the *mer* operon which provides them with resistance mechanisms which are effective in preventing mercury damage to their cells (Monachese et al. 2012; Osborn et al. 1997). Heavy-metal and antibiotic resistance genes are often encoded together on the same plasmid. Therefore, with frequent exposure to heavy metal and/or antibiotics a selective pressure exists to keep the plasmid in the microbiota of the intestinal tract (Monachese et al. 2012).

125. Human gut microbiota is also excreted into sewage and waste water, where it is removed through standard waste treatment processes, prior to the water being released back into the environment.

7.2 Presence of related bacterial species in the receiving environment

126. About 200 recognized O serogroups of *V. cholerae* are known, however only serogroup O1 and O139 have been associated with severe disease and cholera pandemics. Intestinal and /or extraintestinal infections with non-O1 and -O139 serogroups or non-toxigenic O1 strains are rarely found and seem to be of little clinical significance.

127. Wild-type cholera toxin producing *V. cholerae* exist in the Australian environment. The first reported case of cholera acquired from the Australian aquatic environment occurred in

1977. During investigations carried out since then, *V. cholerae* has been isolated from river systems in the east and north west of Australia, including the Rockhampton, Brisbane, Lismore and Sydney areas (Desmarchelier et al. 1995b).

128. Non-O1 and non-O139 strains are more frequently isolated from rivers and estuarine areas than O1 and O139 strains and most environmental O1 strains are non-toxigenic (Reidl & Klose 2002).

7.3 Presence of similar genes and encoded proteins in the environment

129. Mercury resistance (*mer* operon) is the most wide-spread of all antimicrobial resistance determinants. It occurs naturally in a wide variety of Gram-negative and Gram-positive bacterial genera, persisting in different environments. Expression of the *mer* operon allows bacteria to survive in waters contaminated with mercury (Hobman et al. 2005). In most Gram-negative bacteria, the *mer* operon is carried on mobile genetic elements, such as transposons and plasmids, facilitating horizontal gene transfer (Brown et al. 2002).

130. The *mer* operon can be naturally spread via horizontal gene transfer from one bacterium to another. Natural exchange of the *mer* operon, and resultant mercury resistance, between bacterial populations is very common (Liebert et al. 1999b), particularly where the presence of mercury contamination provides positive selection pressure (Boyd & Barkay 2012; Freedman et al. 2012; Moller et al. 2014).

131. Certain members of the human gut microbiota such as lactobacilli naturally carry the *mer* operon (Monachese et al. 2012; Osborn et al. 1997). Therefore, proteins expressed by the *mer* operon are potentially expressed inside the human gastro intestinal tract by the bacterial species naturally colonising the human gut.

132. The *mer* operon is an inducible operon and it is only activated in the presence of mercury ions. However, a low level of merR expression may occur even in the absence of mercury. In laboratory conditions, the *mer* operon is activated when bacteria are exposed to at least 20 µM of mercuric chloride (Viret *et al* 2004).

133. The *mer* operon used in the GM vaccine strain is from the bacteria *S. flexneri*, which is present in the Australian environment. Infections with *S. flexneri* are reported every year in Australia, though the rate of infection is low compared to developing countries (The Department of Health 2013). Transmission of a multiple drug resistance plasmid, which also contains the *mer* operon, between *S. flexneri* and another *Vibrio* species, *Vibrio comma*, has been observed to occur naturally by conjugation of these two bacterial species (Kuwabara et al. 1963). The *mer* operon is already present in microbial communities and evidence suggests it can transfer naturally between bacterial species including to *Vibrio* species.

Section 8 Relevant Australian and international approvals

8.1 Australian approvals

134. Commercial release of this GM *V. cholerae* vaccine strain as a human vaccine (formerly known as Orochol®) was previously approved in Australia by the Genetic Manipulation Advisory Committee (GMAC), the TGA and subsequently by the Gene Technology Regulator under licence DIR 033.

135. On 24 November 1999, GMAC issued an approval for an Activity with the Potential for Unintended Release of GMOs (UR-4) for the commercial release of Orochol®.

136. With the implementation of the gene technology legislation in 2001 and in accordance with section 190 of the Act, a 'deemed' licence for Orochol® was issued to CSL on 18 June 2001 which was valid for two years until 21 June 2003.

137. CSL then applied for continued commercial release of Orochol®. After reassessing the application, the Gene Technology Regulator issued DIR 033 to CSL on 20 June 2003. This licence was subsequently surrendered at the licence holder's request on 14 September 2010.

138. In 2009 the applicant, PaxVax, acquired a worldwide exclusive licence to the GM vaccine strain from the original developers, the Center for Vaccine Development at the University of Maryland, Baltimore.

139. The GM vaccine is now manufactured by PaxVax under the trade name PXVX0200. Although this is the same vaccine strain as used in Orochol®, the applicant is conducting the clinical trials to confirm the safety and efficacy of the newly manufactured product PXVX0200.

140. Since the acquisition of the licence, PaxVax has conducted a Phase I study to evaluate the safety and immunogenicity of the GM vaccine in healthy adults. Initial results confirmed that the newly manufactured product has a similar safety and immunogenicity profile as the previously manufactured product (Chen et al. 2013b).

8.1.1 Approvals by other government agencies

141. Orochol® was registered as a prescription medicine by the TGA under the *Therapeutic Goods Act 1989* on 17 April 2000. Applications to register a pharmaceutical are subjected to a rigorous evaluation by the TGA. The evaluation concluded that Orochol® was "of acceptable standard of quality, safety and efficacy for registration as a prescription medicine for the proposed human use."

142. A condition of the TGA approval was the preparation of a post-marketing report each year for three years from the date of approval. This post-marketing report required inclusion of any suspected adverse drug reactions.

143. The vaccine Orochol® was sold in Australia between 2 September 2000 and 20 June 2003 with over 80,000 doses distributed. As reported by CSL Ltd no vaccine was sold while the GMO was authorised under Licence DIR 033.

144. In 2008 CSL voluntarily surrendered the TGA registration for Orochol®.

145. The Database of Adverse Event Notifications managed by the TGA notes that only two suspected adverse events have been reported in Australia in relation to Orochol®. One patient developed nausea (though the patient had received Hepatitis A and B vaccines at the same time); and one patient developed extensive oropharyngeal *Herpes Simplex virus* but recovered without sequelae (received typhoid vaccine at the same time and was also receiving immune suppressants) (http://www.tga.gov.au/daen/daen-report.aspx). Reports of adverse events have led to the WHO recommending that Orochol® and live typhoid vaccine should not be administered simultaneously (WHO recommendations 2014).

146. The Australian Quarantine and Inspection Service (AQIS) previously provided CSL with an import permit for Orochol®. The permit was valid until 22 April 2004. PaxVax will be seeking a new import permit for the GM vaccine from the Department of Agriculture.

8.2 International approvals of GM Cholera Vaccine

147. The GM vaccine was previously registered for commercial sale (under the trade name Orochol®) in several countries, including Switzerland, Austria, Finland, Canada, Australia, New Zealand, Sri Lanka, the Philippines, and several South American countries.

148. Orochol® was first registered in Switzerland in 1994. Worldwide, over 220,000 doses were sold between 1998 and 2000, with over 500,000 doses sold in total.

149. During the period of 1998 to 2000, four adverse events were reported, three from Germany and one from Switzerland: one case of Guillain-Barre syndrome classed as not

causally related to the vaccine; one case of alopecia classified as remotely causally related to the vaccine; one case of gastroenteritis and vomiting; and one case of facial angioedema. While the latter two events were both causally related, the patients recovered fully.

150. Orochol® was sold in New Zealand under legal exemption from 1998 and approval for the vaccine was given in March 2000. However, it was withdrawn from the New Zealand market by the Environmental Risk Management Authority in June 2000 due to the vaccine being a genetically modified organism and therefore its distribution breached the *Hazardous Substances and New Organisms Act 1996* (HSNO Act). As communicated by the applicant, the importer of the vaccine failed to meet the requirements of the HSNO Act.

151. Production of Orochol® was stopped in 2004 and it is no longer available for sale. The manufacturer stopped production of the vaccine voluntarily, due to business considerations (WHO 2013).

152. Orochol® vaccine was approved for use in the following countries:

Table 3 Summary of International Releases

Registered in:	Registration No.	Year of Registration
Argentina	43 444	1994
Bolivia	II-16455/97	1997
Canada	02229525	1996
Colombia	M-12791	1999
El Salvador	F004602022000	2000
Finland	205/842/96	1996
Guatemala	PF-21,413	1999
Honduras	M-07261	1999
New Zealand	N/A	2000
Panama	46775	1997
Peru	E-8912	1995
Philippines	BR-361	1997
Sri Lanka	DR-010887	1998
Switzerland	555	1994
Switzerland	591	1995
Venezuela	SR.97.0326	1998

8.3 WHO review of Orochol® vaccine

153. A review of data from clinical trials of Orochol® vaccine, composed of the same GM *V. cholerae* strain as PXVX0200, was conducted by WHO in 2001. This included data from a field efficacy study involving 67,508 subjects that was conducted in North Jakarta. This review concluded that extensive trials in a number of countries in Africa, Asia and Latin America have established that this single dose vaccine has been shown to be safe and immunogenic in a series of Phase I and II randomized, controlled clinical trials in adults and children (WHO 2013).

Chapter 2 Risk assessment

Section 1 Introduction

154. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 4). Risks are identified within the context established for the risk assessment (see Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

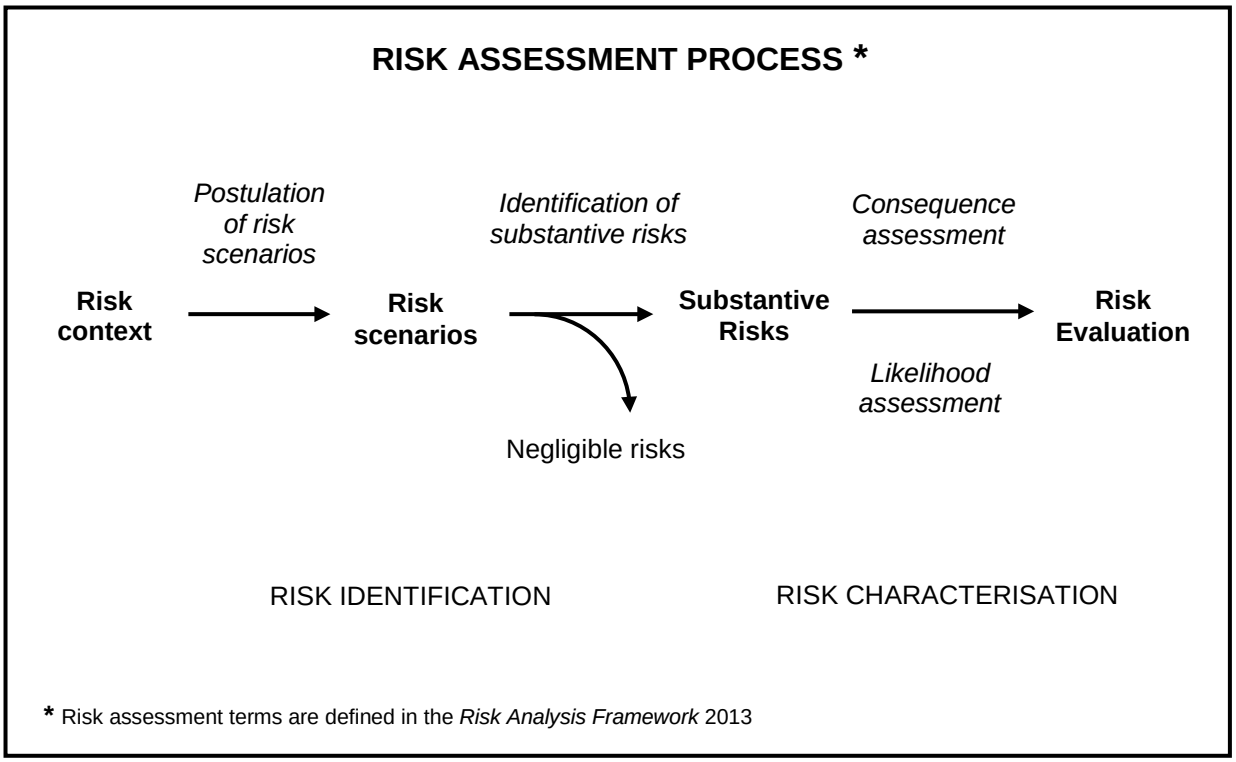

Figure 4. The risk assessment process

155. Initially, risk identification considers a wide range of circumstances whereby the GMO, or the introduced genetic material, could come into contact with people or the environment. Consideration of these circumstances leads to postulating plausible causal or exposure pathways that may give rise to harm for people or the environment from dealings with a GMO (risk scenarios).

156. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified for further assessment when a risk scenario is considered to have some reasonable chance of causing harm. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

157. A number of risk identification techniques are used by the Regulator and staff of the OGTR, including checklists, brainstorming, reported international experience and consultation (OGTR 2013). In conjunction with these techniques, risk scenarios postulated in previous RARMPs prepared for licence applications of the same and similar GMOs are also considered.

158. Substantive risks (*i.e.* those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments.

Section 2 Risk Identification

159. The following factors are taken into account when postulating relevant risk scenarios:

- the proposed dealings, which may be to conduct experiments, develop, produce, breed, propagate, grow, import, transport or dispose of the GMOs, use the GMOs in the course of manufacture of a thing that is not the GMO, and the possession, supply and use of the GMOs in the course of any of these dealings
- the proposed limits;
- the proposed controls;
- characteristics of the parent organism(s);
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential effects of the introduced gene(s) and gene product(s) expressed in the GMOs;
- potential exposure to the introduced gene(s) and gene product(s) from other sources in the environment;
- the environment at the site(s) of release; and
- clinical management practices for the GMOs.

160. Seven risk scenarios were postulated and evaluated. They are summarised in Table 4, where circumstances that share a number of common features are grouped together in broader risk categories. In the context of the control measures proposed by the applicant, and considering both the short and long term, none of the risk scenarios were identified as a risk that could be greater than negligible. Therefore, they did not warrant further detailed assessment. More detail of the evaluation of these scenarios is provided later in this section.

161. As discussed in Chapter 1, Section 3, the Therapeutic Goods Administration (TGA), the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) all have roles in ensuring the safety of participants under the *Therapeutic Goods Act 1989,* and the use of a therapeutic good in a clinical trial must be in accordance with the *National Statement on the Ethical Conduct in Research Involving Humans* (National Health and Medical Research Council 2013). Therefore, risk scenarios in the current assessment focus primarily on risks posed to people other than those participating in the clinical trial, and to the environment.

Table 4 Summary of risk scenarios from dealings with GM Cholera Vaccine

Risk category	Risk scenario		Substantive risk	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.1 Production of a substance toxic to people or other organisms as a result of the genetic modification	1. Exposure of people and other organisms to the GM cholera vaccine	• Toxicity in people and other organisms due to expression of proteins from the *mer* operon or as a result of the gene deletions	No	• The *mer* operon does not encode any toxins or proteins known to cause significant adverse effects. • Loss of cholera toxin expression and loss of haemolysin expression does not change the expression of other genes or result in the production of any novel proteins or other substances. • The *mer* operon is naturally present in the gut flora of human GI tract and is only expressed in the presence of mercury. • Previous clinical trials have not reported any significant adverse effects which can be attributed to the loss of toxin and haemolysin expression or to the proteins encoded by the *mer* operon. • The limits and controls on the trial minimise the likelihood of people or animals being exposed due to unintentional release: - Transport of vaccine stocks would be according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*; - Storage would be at secure clinical sites; - Disposal would be through the clinical waste stream; - Procedures have been proposed to ensure all vaccine stocks would be accounted for. • Vaccine administration would be conducted by trained medical professionals wearing appropriate protective clothing and other precautions would be taken to avoid exposure. • Individuals with conditions which may make them more susceptible to disease, such as pregnant or nursing women, people with medical history of gastrointestinal disorders, immunodeficient or immunosuppressed persons, would be excluded from participating in the trial.

Risk category	Risk scenario		Substantive risk	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.2 Increased disease burden as a result of the genetic modification	2. Exposure of clinical staff to the GM cholera vaccine resulting in infection and disease	• Increased disease burden	No	• Bacteria containing plasmids encoding the *mer* operon are widely distributed in microbial communities, including lactobacilli species which naturally colonise the human gut. • The GM vaccine has been modified to prevent production of the cholera toxin and haemolysin proteins and is unable to cause disease. • A large dose ($8x10^8$ CFU) of vaccine (in appropriate buffer) is required to establish a transient infection and generate an immune response. • Without the bicarbonate buffer, the GM vaccine is killed by the acids found in the human stomach. • GM cholera vaccine is susceptible to commonly used antibiotics. • Vaccine administration would be conducted by trained medical professionals wearing appropriate protective clothing and other precautions would be taken to avoid exposure. • Storage and disposal of the GM vaccine (including the clean-up of spills) would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.
	3. Exposure of contacts of trial participants (household contacts and animals) to GM cholera vaccine strain resulting in infection and disease	• Increased disease burden	No	• *V. cholerae* does not cause disease in animals or organisms other than humans. • The GM vaccine has been modified to prevent production of the cholera toxin and haemolysin proteins and is unable to cause disease. • After vaccination, less than one third of clinical trial participants may shed a small amount of the GM vaccine for about a week. • Transmission route is via ingestion of food or water contaminated with large amounts of infectious cholera bacterium. • Vaccine strain was not detected in household sewage samples of vaccinated individuals. • Sewage treatment is common practice in most Australia cities. • Very limited transmission of vaccine strain to close contact / family members of trial participants was reported in previous clinical trials. • Trial participants would be instructed to practice good hygiene.

Risk category	Risk scenario		Substantive risk	Reason
	Pathway that may give rise to harm	Potential harm		
	4. Exposure of people or animals to the GM cholera vaccine due to unintentional release resulting in infection and disease	• Increased disease burden	No	• Exposure is highly unlikely to result in increased disease symptoms for the same reasons detailed in scenarios 1 -3 above. • GM cholera vaccine without the bicarbonate buffer is very susceptible to hot and dry conditions (needs refrigeration for storage) as well as stomach acid. • The limits and controls on the trial minimise the likelihood of people or animals being exposed due to unintentional release: - Transport of vaccine stocks would be according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*; - Storage would be at secure clinical sites; - Disposal would be through the clinical waste stream; - Spills will be decontaminated appropriately - Procedures have been proposed to ensure all vaccine stocks would be accounted for.
Section 2.3 Unintended changes in bacterial characteristics	5. Changes to the characteristics of the GM cholera vaccine strain resulting from genetic modifications	• Increased disease symptoms • Altered host range	No	• Safety of the GM cholera vaccine strain has been extensively tested in several clinical trials with no significant adverse events reported. • The GM cholera vaccine was previously commercially distributed in Australia and internationally with no reports of harm. • The genome of the GM vaccine has been fully sequenced and characterised. • Genetic stability of the GM vaccine has been investigated in several different studies and was found to be stable. • After vaccination, less than a third of clinical trial participants would shed a small amount of vaccine strain for about a week. • Vaccine strain was not detected in testing of household sewage samples of vaccinated individuals. • Sewage treatment or use of septic tanks is common practice in Australia. • Plasmid encoding the *Mer* operon is widely distributed in microbial communities living in their natural habitats. • The *Mer* operon is naturally passed from one microorganism to the next and is not known to increase the pathogenicity or toxicity of the receiving organism.

Risk category	Risk scenario		Substantive risk	Reason
	Pathway that may give rise to harm	Potential harm		
Section 2.4 Horizontal transfer of genes or genetic elements	6. Horizontal gene transfer resulting in movement of genetic material to or from the GM vaccine strain.	• GM cholera vaccine strain reverting to the pathogenic form • Adverse effect on related bacterial species • Receiving organism gaining selective advantage for spread and persistence in the environment.	No	• Pathogenic and non-pathogenic *V. cholerae* are present in the Australian environment. • Horizontal Gene Transfer (HGT) resulting in the restoration of cholera toxin expression would not increase the pathogenicity of the microorganism above that of the parent strain. • Mating experiments carried out under ideal circumstances were unable to transfer the introduced genetic material contained on the chromosome. • GM cholera vaccine strain is a non-permissive host for the most common cholera bacteriophage. • Persons with known gastrointestinal issues or a history of such issues would be excluded from the trial. • Plasmids encoding the *mer* operon are widely distributed in microbial communities and are naturally passed between microorganisms. This includes lactobacilli species which naturally colonise the human gut. • The introduced *mer* operon is incorporated into the chromosome of the GM vaccine strain, and is very stable in comparison to plasmids which can be transferred much more easily.
Section 2.5 Unauthorised activities	7. Use of the GM cholera vaccine outside the licence conditions (non-compliance)	Potential adverse outcomes mentioned in Sections 2.1 to 2.4	No	• The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs and also requires consideration of the suitability of the applicant to hold a licence prior to the issuing of a licence by the Regulator.

2.1 Production of a substance toxic or allergenic to people or toxic to other organisms

162. Toxicity is the adverse effect(s) of exposure to a dose of a substance as a result of direct cellular or tissue injury, or through the inhibition of normal physiological processes (Felsot 2000).

163. A range of organisms may be exposed directly or indirectly to the proteins encoded by the introduced genes. Trial participants would be intentionally exposed to the GM cholera vaccine. Clinical staff administering the vaccines or other staff handling the vaccines during transport, storage and disposal may be accidently exposed through a spill or contact with contaminated items. People and other organisms may be exposed to the GM bacteria if it is shed by trial participants. Transmission and infection as a result of bacterial shedding is considered in Risk Scenario 3.

Risk Scenario 1. Exposure of people and other organisms to the GM cholera vaccine resulting in toxicity due to expression of proteins from the mer operon or as a result of the gene deletions.

164. Proteins encoded by the *mer* operon are discussed in Chapter 1, Section 6.3.2 and 6.3.3. None of these proteins are known to have a toxic effect or have ability to cause significant

adverse effects. Similarly, loss of cholera toxin expression and loss of haemolysin expression does not change the expression of other genes or result in the production of any novel toxic proteins or other toxic substances.

165. As discussed in Chapter 1, Section 6.3.3, mercury resistance is the most widespread bacterial resistance determinant found in microbial communities. Natural exchange of the *mer* operon, and transfer of mercury resistance, between bacterial populations is commonplace (Liebert et al. 1999b). The proteins expressed by the *mer* operon help bacterial species to protect themselves from mercury damage, and metabolise any mercury ions present in their surrounding environment (Monachese et al. 2012; Osborn et al. 1997).

166. Bacterial species such as lactobacilli are part of normal human gut flora and they naturally carry the *mer* operon. The *mer* operon is an inducible operon and, other than merR, the encoded proteins are only expressed when the host organism comes in contact with mercury ions above a threshold level. Therefore, the majority of the proteins encoded by the *mer* operon would not normally be expressed inside a human host. However, if a person consumes food (e.g. seafood) contaminated with mercury, all the Mer proteins would be expressed by the lactobacilli and other gut flora. The presence of proteins encoded by the *mer* operon is not known to cause toxic or adverse effects to its host or to the organisms present in its surrounding environment (Freedman et al. 2012).

167. Similarly, the proteins encoded by the *mer* operon in the GM vaccine would not be expressed within people or other organisms unless they are exposed to levels of mercury above the activation threshold. If expressed, the Mer proteins would not cause toxic or adverse effects.

168. A large number of clinical trials of the GM cholera vaccine (previously known as Orochol®) involving thousands of subjects have been carried out. These trials have demonstrated that this vaccine is safe for human use with a very low incidence of adverse reactions such as nausea, vomiting or angioedema. There are no reports of toxic or allergic reactions in vaccine recipients which can be directly attributed to the proteins encoded by the *mer* operon (see Table 2 for details).

169. The proposed limits and controls of the trial (Chapter 1, Section 4.1 and 4.2) will minimise the likelihood of exposure of people not enrolled in the trial and other organisms in the environment to the GM vaccine. Individuals with conditions which may make them more susceptible to disease, such as pregnant or nursing women, people with a medical history of gastrointestinal disorders, and immunodeficient or immunosuppressed persons would be excluded from participating in the trial.

170. Human contact with the GM vaccines prior to and during the administration of the vaccine would be limited to trained, authorised staff who can meet the above exclusion criteria. The staff will be wearing appropriate personal protective equipment, including a laboratory coat, gloves and safety glasses. The proposed trial sites are located within hospitals so access to the general public would be minimised. Storage, transport and disposal of the GM vaccine, and contaminated waste would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*.

171. ***Conclusion***: The risk of toxicity resulting from exposure of people or other organisms to the proteins encoded by the introduced genes is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.2 Increased disease burden from the GM cholera vaccine

172. The GM cholera vaccine strain is not able to cause disease. It was previously used in a traveller's vaccine (trade name Orochol®) with over 80,000 doses sold in Australia. Only two adverse events are reported in the TGA Database of Adverse Event Notifications - medicines

(www.tga.gov.au/daen/daen-report.aspx). Both were minor in nature and were not directly related to the vaccine.

173. Trial participants would be intentionally exposed to the GM cholera vaccine. Clinical staff administering the vaccines or other staff handling the vaccines during transport, storage and disposal may be accidently exposed through a spill or contact with contaminated items. People and other organisms may be exposed to the GM bacteria if it is shed by trial participants.

174. An increased disease burden could occur due to an increase in disease symptoms, or inappropriate immune response to GM bacteria, as a result of expression of the proteins encoded by the introduced genes. An inappropriate immune response would be considered to be an abnormal/unintended increase or suppression of the immune response, or an allergic response. Pathways that could lead to an increased disease burden from the GM cholera vaccine include:

- exposure of clinical staff to the GM cholera vaccine, leading to infection and protein expression;
- exposure of contacts of trial participants (household contacts and animals) to the GM cholera vaccine strain, leading to bacterial infection and protein expression;
- unintentional release of the GM cholera vaccine leading to infection and protein expression in other people or animals.

These are discussed below.

Risk Scenario 2. Exposure of clinical staff to the GM cholera vaccine resulting in infection and increased disease burden

175. As discussed in Risk Scenario 1, the majority of the Mer proteins are not expressed in the absence of mercury, and if expressed they would not result in toxicity or adverse immune reaction as they are already expressed by bacteria naturally present in the human intestinal tract and humans are already exposed to these proteins.

176. The GM cholera vaccine has been modified such that it is not able to produce the two main pathogenic determinants of cholera: cholera toxin and haemolysin. Therefore, the GM vaccine is not able to cause disease. A large dose of vaccine ($8x10^8$ CFU) in a bicarbonate buffer is required to establish the transient infection necessary to generate an immune response. The GM cholera vaccine without the bicarbonate buffer is very susceptible to the acid content of the human stomach. Therefore, exposure to a small amount of vaccine without the buffer is highly unlikely to result in infection, and the vaccine is not able to cause disease.

177. Additionally, the GM cholera vaccine is susceptible to commonly used antibiotics. The applicant has indicated that in an unlikely event of accidental exposure, antibiotic therapy would be provided to exposed personnel, if clinically indicated.

178. Clinical staff administering the vaccine, or other staff handling the vaccines during transport, storage and disposal, may be accidently exposed through a splash to the eye or mouth, a spill, patient rejection (vomit) or contact with items that have been contaminated with the GM cholera vaccine.

179. The phase III clinical trial protocols and the pharmacy manual documents outline precautions to be taken by clinical staff when administering the vaccine. These documents prescribe that when handling the GM bacteria, staff would be conducting the dealings in accordance with ICH-GCP guidelines, which, along with other precautions, state that staff must wear appropriate personal protective clothing.

180. ICH-GCP guidelines also include routine monitoring of the conduct of the study at the clinical trial sites and associated pharmacies. This monitoring assesses, among other things,

compliance with the protocol approved by the HREC for each site, and correct handling of clinical trial material (including correct storage, preparation and disposal by the site pharmacy).

181. The staff conducting the trial would be appropriately trained and would be instructed in handling and disposal of waste such as:

- destroying left over liquid vaccine by chemical methods such as 10% bleach or 70% isopropyl alcohol before disposal at the clinical site following institutional procedures for the disposal of biohazardous material;
- discarding waste generated during the conduct of the study such as disposable cups and empty vaccine sachets into appropriate biohazard containers, and disposing of the waste at the clinical site following institutional procedures for the disposal of biohazardous material; and
- disposing unused study vaccine at the clinical site following institutional procedures for the disposal of biohazardous material, such as incineration.

182. These measures are consistent with the Regulator's Guidelines for the Transport, Storage and Disposal of GMOs.

183. The applicant has proposed a spill management plan and procedures to respond to an accidental exposure to the vaccine (Chapter 1, Section 4.2). This is likely to minimise accidental exposure of clinical staff to the GM cholera vaccine and minimise consequences of any exposure.

184. ***Conclusion*:** The potential for the GM cholera vaccine to increase disease burden following infection of clinical staff, resulting in increased disease symptoms or an inappropriate immune response due to the expression of introduced genes, is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Risk Scenario 3. Exposure of contacts of trial participants (household contacts and animals) to GM cholera vaccine strain resulting in infection and increased disease burden

185. Wild-type *V. cholerae* strains have been isolated from fish and shellfish living in waters contaminated by the bacteria, but these animals are carriers and are not affected by the bacteria. There is no evidence that the bacteria can cause disease in animals even in areas where cholera is endemic and outbreaks are common (Shears 1994).

186. Commonly used laboratory animals are resistant to colonisation and infection by *V. cholerae*. The bacteria will not colonise adult mice or rabbits without procedures such as sealing the ano-rectal canal of adult mice and tying off loops of the rabbit small bowel. Diarrhoea caused by *V. cholerae* can be induced in neonatal mice, but the animals are susceptible only for a short time after birth (Kaper et al. 1995).

187. As discussed in Chapter 1, Section 5.4, toxin co-regulated pilli (TCP) are the primary host range determinants for pathogenic *V. cholerae* (Kaper et al. 1995) as it mediates bacterial cell binding to host cells and therefore allows for colonisation of the host gut. The TCP in the GM vaccine has not been modified. Haemolysin is not considered a host range factor for *V. cholerae* (Stonehouse et al. 2008). Similarly loss of cholera toxin and expression of the *mer* operon is not expected to change the host range of the GM vaccine. Therefore, the GM vaccine would not be expected to colonise or have an adverse effect on animals or any other organisms in the environment, other than humans.

188. As discussed in Risk Scenario 1 and Risk Scenario 2, the GM *V. Cholerae* is not toxic to humans and cannot cause disease. Deletion of the cholera toxin and haemolysin genes means that exposure to the GM vaccine strain would not result in an increased disease burden in humans.

189. Toxigenic *V. cholerae* are excreted by untreated patients for up to 2 – 3 weeks, but very rarely for longer periods (Dizon et al. 1967; Kaper et al. 1995). Data from clinical trials have demonstrated that only 30% or fewer GM cholera vaccine recipients excrete detectable numbers of the GM bacteria for a maximum of 7 days (Chen et al. 2013a; Kotloff et al. 1992; Lagos et al. 1999; Levine et al. 1988; Simanjuntak et al. 1993). The maximum observed number of GM bacteria excreted was 200 bacteria per gram of faeces on the peak day of excretion of GM bacteria, with significant reductions on subsequent days.

190. Therefore, during the peak day of excretion of GM bacteria, approximately 4×10^4 live GM bacteria would be excreted by less than one third of the recipients. As discussed in Chapter 1, Section 5.4, the main transmission route for *V. cholerae* is via ingestion of food or water contaminated with large amounts of infectious cholera bacterium. The infectious dose for toxin-producing *V. cholerae* can be as high as 10^{11} CFU when given without food. However, when the bacteria are ingested with food the infectious dose is 10^6 CFU (Kaper et al. 1995).

191. A large dose (8×10^8 CFU) of vaccine in a bicarbonate buffer is required to establish a transient infection and generate an immune response. The GM Cholera vaccine without the bicarbonate buffer is very susceptible to higher temperature as well as acids of human stomach. Therefore, exposure to small amount of vaccine without the buffer is highly unlikely to result in infection and vaccine is not able to cause disease.

192. A study involving 303 children (24 to 59 months-old) who received the GM vaccine, and their household contacts (including young siblings) found that the transmission rate between the vaccinee and their household contacts was minimal (Simanjuntak et al. 1993). Transmissibility of the vaccine was evaluated by culturing stool samples as well as testing blood samples for serocoversion in unvaccinated household contacts who provided samples for testing. The vaccine was isolated from stool of only 1 of 177 (0.66%) unvaccinated family contacts (a mother). One sibling of the vaccinated children (1.5%) manifested seroconversion and 4 siblings (6%) showed an increase in vibriocidal antibody levels (Simanjuntak et al. 1993). Overall the vaccine was found to be safe and immunogenic with no significant adverse reactions observed in vaccinated children or their household contacts (Simanjuntak et al. 1993).

193. In the same study, in 97 households of people who had received the vaccine, Moore swabs (4 cm thick gauze rolls attached to a nylon string) were used in an attempt to isolate GM bacteria from household effluent (Simanjuntak et al. 1993). Samples were taken from where the household effluent entered the sewerage drains or from toilets. The vaccine strain was not isolated from any of the samples taken. Non-O1 *V. cholerae* (i.e. strains other than the vaccine strain) were isolated from 46 of the samples.

194. In Australia, treatment of wastewater (including sewage) is required as per State and Territory regulations. Commercial wastewater treatment is carried out in local government areas where this clinical trial is proposed to be conducted (refer to Table 1) (Queensland 2009; Queensland 2012; South Australia 1993; South Australia 2009; Victoria 2000; West Australia 2004). This would significantly limit the chances of genetically modified bacteria entering into the environmental waters. Septic tanks are used in some local government areas where commercial wastewater treatment is not available and septic tanks are required to be maintained in accordance with State and Territory regulations. This means that untreated sewage should not leak from the septic tank. Nevertheless, septic tank leakage can occur, and it is possible that persons servicing the septic tank or working with the surrounding soil may come into contact with the GM bacteria. However, this is not considered a plausible pathway to harm for the following reasons:

- only a few trial participants are likely to be using the septic tanks and considering only 30% of the vaccine recipients are likely to shed the GM bacteria, not all of vaccine recipients using the septic tanks are likely to be shedding the GM bacteria. Therefore, only small amount of GM bacteria is likely to enter into septic tanks;

- once in the septic tank, the small amount of GM bacteria would be significantly diluted, the GM bacteria are not likely to replicate very well outside the human host and are not likely to be able to compete with other bacterial species present in the septic tank. Therefore, the GM bacteria would not be able to persist in large numbers or survive for very long in the septic tank;
- during the previous studies it has been demonstrated that GM bacteria could not be isolated from the household effluent and does not survive for very long in the soil (Simanjuntak et al. 1993; Viret et al. 2004); and
- ingestion of a small amount of GM bacteria without the buffer is highly unlikely to result in infection and vaccine is not able to cause disease.

195. A possible route of exposure of non-trial participants to the GM vaccine organisms is direct contact with a vaccine recipient. However, in areas where sewerage systems are in place and clean running water is available for washing hands, cholera is rarely transmitted to family members by direct contact (WHO 2013).

196. The applicant has stated that trial participants would be educated and instructed to:

- wash hands with soap and water after using the bathroom and always before handling food or eating;
- keep hands and any unclean items, or items used for toilet purposes, away from the mouth, eyes, ears, nose, and wounds; and
- avoid use of shared or unclean eating utensils, drinking cups, towels and handkerchiefs.

197. Parents/caregivers of child participants would be instructed to use disposable nappies where necessary, and enclose the used nappies in two sealed plastic bags prior to disposing in standard household waste streams, for the 3-4 week period post-vaccination. These instructions would be included within the informed consent for the paediatric study.

198. In summary, standard hygiene and sewage treatment practices are sufficient to minimise exposure of contacts of trial participants (household contacts and animals) to the low level of GM *V. cholerae* excreted by trial participants.

199. ***Conclusion:*** The potential of GM *V. cholerae* to increase disease burden following exposure of close contacts of trial participants, resulting in increased disease symptoms or an inappropriate immune response due to the expression of introduced genes, is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Risk Scenario 4. Exposure of people or animals to the GM cholera vaccine due to unintentional release resulting in infection and increased disease burden

200. As discussed in Risk Scenario 1 and Risk Scenario 2, the GM vaccine does not cause disease and is not toxic to humans, due to deletion of parts of the cholera toxin and haemolysin genes. Exposure of clinical staff to the GM vaccine would not result in an increased disease burden in humans.

201. As discussed in Risk Scenario 3, *V. cholerae* does not cause disease in organisms other than humans and the GM vaccine would not colonise or have an adverse effect on animals or any other organisms in the environment, other than humans.

202. An unintentional release would include spills outside of the clinical trial sites. This could occur as a spill during import, storage, transport or disposal.

203. The applicant has stated that the sealed vaccine sachets (sealed primary containers) would be transported (with ice packs) within sealed and unbreakable secondary containers

marked with a label to indicate that they contain GMOs. The outside of the package would include the address and phone number of the relevant contact person.

204. The study vaccines and placebos would be shipped from a clinical packaging facility in the United States of America direct to the clinical trial sites in Australia, by commercial couriers specialising in the handling of vaccines and other medicines requiring temperature-sensitive shipping.

205. Any spills occurring in a clinical setting would be disinfected and cleaned according to standard clinical procedures. Spills outside of clinical facilities (i.e. during transport, storage or disposal) would be disinfected and contained according to the requirements of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. This includes requirements that the GMOs be destroyed via a method appropriate for the GMO type, that all packages of GMOs are accounted for, and that the incident is reported to the Licence holder and the Regulator.

206. In addition, the GM cholera vaccine is supplied as lyophilized powder which requires refrigeration to maintain viability during storage. The lyophilized vaccine has reduced capacity to survive in the environment compared to bacteria found in biological specimens. Additionally, without the bicarbonate buffer the GM bacteria would have poor survival in the acid conditions of the human gastrointestinal tract.

207. Disposal of medical waste from the vaccination process would be via the clinical/biohazardous waste stream at the study site. Following administration, used disposable cups and empty vaccine sachets that contained GM vaccine would be immediately placed into infectious waste containers or into bags that would be sealed, and retained for accountability.

208. The waste would be destroyed at the clinical site following standard clinical waste disposal methods such as steam sterilisation or incineration. The *Industry Code of Practice for the Management of Clinical and Related Wastes* details requirements for clinical waste including waste segregation, packaging, labelling, storage, transport and accountability (Biohazard Waste Industry Australia and New Zealand (BWI) 2010).

209. The clinical waste stream is considered appropriate for disposal of the GM vaccine (Queensland 2009; Queensland 2012; South Australia 1993; South Australia 2009; Victoria 2000; West Australia 2004). All unused study vaccine would also be disposed of via the clinical waste stream at the site.

210. ***Conclusion:*** The potential for an unintentional release of GM cholera vaccine to increase disease burden in humans or other organisms is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.3 Unintended changes in bacterial characteristics

211. The GM cholera vaccine has been modified to include the *S. flexneri* mercury resistance (*mer*) operon. This would provide the genetically modified bacteria with a selective advantage in mercury polluted environments.

212. When genes are deleted from or inserted into a genome, there is a possibility that the deletion or insertion may have unintended consequences on the expression of other genes. The gene product of one gene may effect apparently unrelated, multiple phenotypic traits, referred to as pleiotropy (Kahl 2001).

Risk Scenario 5. Changes to the characteristics of the GM cholera vaccine strain resulting from the genetic modifications leading to increased disease burden or altered host range.

213. Although the molecular properties of the GM vaccine are well characterised, there is some possibility that there could be unexpected changes to the characteristics of the GM vaccine as a result of the genetic modifications.

214. As discussed in Chapter 1, Section 6.4.4, the safety of the GM cholera vaccine has been extensively tested in human clinical trials and no significant toxicity or significant adverse events have been reported in any of these studies (See Table 2 for details).

215. The same GM vaccine strain has been widely used for commercial release in Australia and Internationally (Chapter 1, Section 8.1and 8.2), with hundreds of thousands of doses administered worldwide. There have been no reports of significant harm to humans or to the environment, or unexpected characteristics of the GM bacteria, as a result of the genetic modifications.

216. The genome of GM cholera vaccine strain has been fully sequenced and other than the *mer* operon there are no additional genomic sequences (e.g. antibiotic resistance genes) present in the genome as a result of the genetic modification.

217. The genetic stability of the GM cholera vaccine was studied after prolonged storage in a lyophilized state and outgrowth for 16-17 generations. No differences were detected using a wide variety of restriction enzymes and probes (Favre et al. 1996).

218. Likewise, complete genetic stability following immunization and passage through the gut was observed for isolates from the stools of 11 trial participant selected from three independent clinical trials (Favre et al. 1996). Therefore, it was concluded that no genetic rearrangements occurred during transit of the bacteria through the digestive tract either at the *ctxA* deletion site or at the *hlyA* deletion/*mer* insertion site. A probe specific to the *ctxA* gene was unable to bind to DNA from the above isolates, which confirmed that a wild-type *ctxA* gene had not been reacquired by any of the tested isolates (Favre et al. 1996).

219. PaxVax recently conducted further assessment of stability and homogeneity by analysis of 16 individual colonies, generated from the expanded progenitor strain, by PCR. All 16 colonies were positive for the *mer* operon, an indication of the expected high stability of the chromosomally integrated *mer* operon (information provided in DIR 126 Application).

220. In summary, there is no evidence that the genetic modifications have resulted in unexpected changes to the characteristics of the GM vaccine, and the stability of the genetic modifications means that this is not expected to change.

221. Bacterial shedding by the trial participants is considered in Risk Scenario 3 and not all of the trial participants are likely to shed the GM bacteria in their faeces. Fewer than 30% of vaccine recipients excrete, at most, 4×10^4 GM bacteria on the peak day of excretion. Additionally, genetically modified bacteria could not be isolated from sewerage drains near households of vaccine recipients (Kotloff et al. 1992; Lagos et al. 1999; Levine et al. 1988; Simanjuntak et al. 1993)(Chapter 1 Section 6.4.2). Therefore it is not expected that large numbers of genetically modified bacteria would be released into the environment.

222. In Australia, treatment of wastewater (including sewage) is required as per State and Territory regulations. Commercial wastewater treatment is carried out in local government areas where this clinical trial is proposed to be conducted (refer to Table 1) (Queensland 2009; Queensland 2012; South Australia 1993; South Australia 2009; Victoria 2000; West Australia 2004). This would significantly limit the chances of genetically modified bacteria entering into the environmental waters. Septic tanks are used in some local government areas where commercial wastewater treatment is not available and septic tanks are required to be

maintained in accordance with State and Territory regulations. This would significantly limit the chances of genetically modified bacteria entering into the environmental waters. However, it is possible that in areas where there is no commercial wastewater treatment, or due to failure of wastewater treatment or septic tanks, small amounts of the genetically modified bacteria could enter the aquatic environment and persist in the dormant state and/or in association with copepods.

223. Parents/caregivers of child participants would be instructed to use disposable nappies where necessary, and enclose the used nappies in two sealed plastic bags prior to disposing in household waste, for the 3-4 week period post-vaccination. This would contain any GM bacteria shed by the children and would eventually inactivate GM bacteria due to the build-up of heat inside two plastic bags. GM cholera vaccine without the bicarbonate buffer is very susceptible to hot and dry environmental conditions (needs refrigeration for storage) and available scientific evidence suggests that the GM bacterium does not survive very long in soil or in estuarine water.

224. The loss of cholera toxin and haemolysin production would not provide a selective advantage for the persistence of GM bacteria in the environment. Expression of the *mer* operon genes would only provide an advantage if the presence of mercury contamination. However, mercury resistance is the most widespread microbial resistance determinant and therefore other mercury resistant bacterial species will be present in such environments (Brown et al. 2002). These bacteria would also provide a source of plasmids containing the *mer* operon which could be transferred naturally to wild-type *V. cholerae* strains, providing them with the same mercury tolerance as the GM vaccine strain.

225. Additionally, *V. cholerae* does not grow well outside of the human digestive system, therefore it is highly unlikely that expression of the *mer* operon by the genetically modified bacteria would allow them to outcompete other bacterial species present in a mercury polluted environment.

226. Toxigenic and non-toxigenic strains of *V. cholerae* are already present in Australian waterways (Desmarchelier et al. 1995b; Kaper et al. 1995). Small numbers of non-toxigenic GM cholera vaccine strain persisting in some Australian aquatic environments would pose a lower risk than toxigenic wild-type *V. cholerae,* and no risks greater than posed by wild-type non-toxigenic *V. cholerae*.

227. ***Conclusion:*** The potential for unintended changes in bacterial characteristics due to the genetic modification leading to increased disease burden or altered host range is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.4 Horizontal transfer of genes or genetic elements

Horizontal gene transfer (HGT) is the stable transfer of genetic material from one organism to another without reproduction (Keese 2008). All genes within an organism, including those introduced by gene technology, are capable of being transferred to another organism by HGT. HGT itself is not considered an adverse effect, but an event that may or may not lead to harm. A gene transferred through HGT could confer a novel trait to the recipient organism, through expression of the gene itself or the expression or mis-expression of endogenous genes. The novel trait may result in negative, neutral or positive effects.

Risk Scenario 6. Gene transfer to or from the GM vaccine strain resulting in an adverse effect for people or the environment

228. Wild-type *V. cholerae* that produce cholera toxin can be found in the Australian environment. The first reported case of cholera acquired from the Australian aquatic environment occurred in 1977 (Desmarchelier et al. 1995b). During investigations carried out since then, *V. cholerae* has been isolated from river systems in the east and north west of Australia, including the Rockhampton, Brisbane, Lismore and Sydney areas.

229. Most of the cases of cholera reported in Australia are acquired overseas by Australian tourists visiting countries where cholera is endemic. Since 1991, six or fewer cases of cholera have been reported annually in Australia (The Department of Health 2013).

230. For the GM bacteria to regain the ability to produce a functional cholera toxin and therefore regain the ability to cause cholera, they would need to acquire a functional *ctxA* gene, which could occur by gene transfer from *V. cholerae* that produce cholera toxin. The cholera toxin genes are contained in a pathogenicity island on the chromosome of toxigenic strains of *V. cholerae* (Mekalanos 1983; Mekalanos et al. 1983).

231. As discussed in Chapter 1, Section 6.4.3, multiple experiments have been carried out to examine the potential for the GM vaccine to regain a functional cholera toxin gene via horizontal gene transfer. Gene transfer was successful only when the GM cholera vaccine strain was mated with a *V. cholerae* strain containing the P fertility plasmid (Kaper et al. 1994). In this system, gene transfer frequency was 1.3×10^{-8}.

232. The P fertility plasmid is not common in toxigenic *V. cholerae* O1 strains. More than 266 *V. cholerae* O1 strains were examined in three separate studies, and no P plasmids were detected (Kaper et al. 1994). Strains possessing this plasmid are avirulent in animal models (Bartowsky et al. 1990; Sinha & Srivastava 1978).

233. In some strains of *V. cholerae*, the pathogenicity island that contains the *ctxAB* genes also includes sequences that encode a transmissible filamentous bacteriophage (Waldor & Mekalanos 1996b). This bacteriophage is able to transfer the *ctxAB* genes from *ctxAB* positive donor strains to *ctxAB* negative recipient strains in the mouse gastrointestinal tract.

234. The GM cholera vaccine strain and its parent strain 569B, however, are non-permissive hosts for the bacteriophage and produce only defective lysogenic bacteriophages that are unable to reinfect permissive *V. cholerae* strains (Favre et al. 1996). Given the GM vaccine strain is immune to bacteriophage infection and it does not produce functional bacteriophages, bacteriophage mediated gene transfer would be highly unlikely.

235. The most likely place for P fertility plasmid-mediated gene transfer, if it were to occur, is in the intestine of a vaccine recipient whose intestines have been colonised by toxigenic *V. cholerae*. However, persons with the history of gastrointestinal disorders and persons who have travelled to a cholera endemic area in the previous 5 years or who have a history of cholera or enterotoxigenic *E. coli* challenge, would be excluded from the trial.

236. If transfer of a functional *ctxA* gene from a *V. cholerae* strain in the environment to the GM cholera vaccine strain were to occur, this would result in the production of a toxigenic *V. cholerae* that would still be unable to produce haemolysin and would, therefore, be less pathogenic than other toxigenic *V. cholerae* present in the environment.

237. The *mer* operon is common in the environment and is transferred naturally between unrelated bacterial species (Bogdanova et al. 1998). In most Gram-negative bacteria, the *mer* operon is carried on mobile genetic elements, such as transposons and plasmids (Brown et al. 2002). However, in the GM cholera vaccine strain the *mer* operon has been inserted into the bacterial chromosome and is not easily transferred. This reduces the likelihood of gene transfer from the GM vaccine to other micro-organisms (Favre et al. 1996; Ketley et al. 1993b). Therefore, even in areas contaminated with mercury, and where selection pressures favour the presence of the *mer* operon, it is more likely that these genes would be acquired from naturally occurring plasmids containing the *mer* operon than from the GM vaccine.

238. The presence of small numbers of GM bacteria that carry the *mer* operon on their chromosome is therefore highly unlikely to have any adverse impacts on the Australian environment. There have been no reports that expression of mercury resistance genes by bacteria harms the environment.

239. Transfer of the inactivated cholera toxin (*ctxA)* gene or haemolysin (*hlyA*) gene from GM bacteria to toxigenic *V. Cholerae* may lead to replacement of their endogenous *ctxA* or *hlyA* genes, making them non-toxigenic or unable to produce functional haemolysin (respectively). Similarly, for non-pathogenic *V. Cholerae,* horizontal gene transfer is not likely to result in an adverse outcome.

240. ***Conclusion:*** The potential of unintended changes in bacterial characteristics due to horizontal gene transfer is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.5 Unauthorised activities

Risk Scenario 7. Use of the GM cholera vaccine outside the licence conditions (non-compliance)

241. Non-compliance with the conditions of the licence could lead to exposure of people in the environment to the GM cholera vaccine outside the scope of the proposed release. The adverse outcomes that may result are discussed in the sections above. The Act provides for substantial penalties for non-compliance and unauthorised dealings with GMOs.

242. The Act also requires the Regulator to have regard to the suitability of the applicant to hold a licence prior to the issuing of a licence. This includes consideration of the applicant's ability to ensure all persons covered by the licence comply with all the conditions which apply to them. PaxVax have indicated that an Australian company specialising in the conduct of clinical trials will be engaged to oversee the trial on their behalf. An examination of the proposed management of the clinical trial by PaxVax, including their nomination of experienced Australian subcontractors, was part of the Regulator's considerations of the applicant's suitability to hold the licence prior to making a decision in relation to this application.

243. These legislative provisions are considered sufficient to minimise risks from unauthorised activities.

244. ***Conclusion*****:** The potential for an adverse outcome as a result of unauthorised activities is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Section 3 Risk estimate process and assessment of significant risk

245. The risk assessment begins with postulation of potential pathways that might lead to harm to the health and safety of people or the environment due to gene technology, and how it could happen, in comparison to the parent organism and within the context of the proposed activities with the GMOs and the receiving environment.

246. Seven risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether expression of the introduced genes or changes in gene expression due to gene deletion could: result in products that are toxic to people or other organisms; alter characteristics that may impact on the disease burden of GM bacteria, or produce unintended changes in bacterial characteristics. The opportunity for unintended exposure to the vaccine or the GM bacteria it contains, and for gene flow to other organisms, was also considered.

247. A risk is only considered substantive and to warrants further assessment when a risk scenario is considered to have some chance of causing harm as a result of the gene technology. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent a substantive risk and do not advance any further in the risk assessment process.

248. The characterisation of the seven risk scenarios in relation to both the seriousness and likelihood of harm, in the context of the control measures proposed by the applicant, did not

give rise to any substantive risks that warranted further assessment. The principal reasons for this include:

- the products of the introduced *mer* operon genes are not expected to be toxic to humans or other animals, and are already widespread in the environment;
- the genetic modifications make the GMO unable to cause disease in people;
- *V. cholerae* does not cause disease in other organisms;
- previous clinical trials have not reported any significant adverse effects attributed to the loss of toxin and haemolysin expression, or introduction of the *mer* operon genes; and
- transmission of the GMO via shedding during the trial would be minimised through appropriate training of both healthcare workers and trial participants and caregivers.

249. Therefore, any risks to the health and safety of people, or the environment, from the proposed clinical trial of the GM cholera vaccine are considered to be negligible. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[4].

Section 4 Uncertainty

250. Uncertainty is an intrinsic property of risk and is present in all aspects of risk analysis, including risk assessment, risk management and risk communication. Both dimensions of risk (consequence and likelihood) are always uncertain to some degree.

251. Uncertainty in risk assessments can arise from incomplete knowledge or inherent biological variability[5]. For clinical trials, because they involve the conduct of research, some knowledge gaps are inevitable. This is one reason they are required to be conducted under specific limits and controls to restrict exposure to the GMOs and their genetic material in the environment, rather than necessarily to treat a substantive risk.

252. For DIR 126, the possibility of increased disease burden and unintended change to bacterial characteristics was considered in individual risk scenarios. As this GMO has been widely used as a vaccine, no significant areas of uncertainty were identified. Additional data, including any unintended effects observed during this trial and other international studies with the GMO, may be required to assess possible future applications for commercial release of the GM vaccine.

[4] As none of the proposed dealings are considered to pose a significant risk to people or the environment, section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP. However, the Regulator allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

[5] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1> or via Free call 1800 181 030.

Chapter 3 Risk management plan

Section 1 Background

253. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan evaluates and treats substantive risks, evaluates controls and limits proposed by the applicant, and considers general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through licence conditions.

254. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

255. All licences are subject to three conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: section 64 requires the licence holder to provide access to premises to OGTR inspectors and section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

256. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under section 152 of the Act.

Section 2 Responsibilities of other Australian regulators

257. Australia's gene technology regulatory system operates as part of an integrated legislative framework. Other agencies that also regulate GMOs or GM products include Food Standards Australia New Zealand (FSANZ), Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutics Goods Administration (TGA), National Health and Medical Research Council (NHMRC), National Industrial Chemicals Notification and Assessment Scheme (NICNAS) and the Department of Agriculture. Dealings conducted under a licence issued by the Regulator may also be subject to regulation by one or more of these agencies[6].

258. The *Gene Technology Act 2000* requires the Regulator to consult these agencies during the assessment of DIR applications. The *Gene Technology (Consequential Amendments) Act 2000* requires the agencies to consult the Regulator for the purpose of making certain decisions regarding their assessments of products that are, or contain a product from, a GMO.

259. The applicant would require appropriate approval under the *Therapeutics Goods Act 1989* for this proposed clinical trial of the GM vaccine.

260. Human Research Ethics Committee (HREC) assessment and approval is an integral part of the governance structure for clinical trials and is also required before the trial can commence.

[6] More information on Australia's integrated regulatory framework for gene technology is contained in the *Risk Analysis Framework* available from the Office of the Gene Technology Regulator. Free call 1800 181 030 or at <http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/riskassessments-1>

Section 3 Risk treatment measures for substantive risks

261. The risk assessment of risk scenarios listed in Chapter 2, Section 2 concluded that there are negligible risks to people and the environment from the proposed trial of a GM vaccine. The risk scenarios were considered in the context of the scale of the proposed release (up to 1000 trial participants, across clinical sites in Australia, over approximately one year), the proposed containment measures (Chapter 1, Section 4.2), and the receiving environment, considering both the short and the long term. The Risk Analysis Framework (OGTR 2013) which guides the risk assessment and risk management process, defines negligible risks as being of no discernible concern, with no present need to invoke actions for mitigation. Therefore, no conditions are included in the licence to treat these negligible risks.

Section 4 General risk management

262. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, licence conditions have been imposed to limit the release to the proposed size, locations and duration, and to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are detailed in the licence and summarised in this Chapter.

4.1 Licence conditions to limit and control the release

4.1.1 Consideration of limits and controls proposed by PaxVax Pty. Ltd.

263. Chapter 1, Section 4.1 and 4.2 provide details of the limits and controls proposed by PaxVax in their application, which are discussed in the risk scenarios characterised for the release in Chapter 2, Section 2. The appropriateness of these limits is considered further below.

264. The proposed release would be confined to 1000 trial participants in Australia. The clinical trial activities would take place at clinical sites in Queensland, South Australia, Victoria and Western Australia. The applicant has indicated that the trial is likely to be completed within one year. However, if the required number of participants cannot be enrolled within one year, the applicant may request a variation to the licence to extend the period in which the trial could take place. These limits would minimise the exposure of people and animals to the GM bacteria and have been included as licence requirements.

265. Limiting the trial to healthy participants who have no previous history of gastrointestinal disorders would minimise the likelihood of adverse effects and the possibility of horizontal gene transfer, and consequently minimises shedding of GM bacteria. Education of staff and trial participants on general hygiene and waste disposal should further minimise the potential for transmission of the GM bacteria to the environment.

266. Exclusion of individuals at risk of adverse effects from exposure to the GM vaccine would reduce the possibility of complications requiring medical treatment and excessive shedding of the GM vaccine into the environment. These include people with immunodeficiencies, women who are pregnant or breastfeeding and children under two years of age. These exclusion criteria have been included as licence requirements.

267. Vaccination would be performed by trained nurses and/or physicians at clinical facilities in accordance with the *World Health Organisation Standard Precautions in Health Care* (World Health Organisation 2007) and the *International Conference on Harmonisation Good Clinical Practice Guidelines* (ICH 1996). The WHO standard precautions detail appropriate hygiene, personal protective equipment and decontamination procedures to prevent direct contact with the GM vaccine. These practices and procedures would minimise exposure of people handling the GM vaccine as part of the trial and have been included as licence requirements.

268. The applicant has proposed standard infection control practices and procedures that minimise exposure to the GM vaccine. Storage and transport, including any waste or samples containing the GM bacteria, would be required in accordance with relevant regulations. These practices and procedures would minimise exposure of other people and the environment to the GM vaccine and have been included as licence requirements.

269. The applicant has stated that all waste would be disposed of in accordance with standard clinical waste disposal practices. These practices and procedures would minimise exposure of other people to the GM vaccine and have been included as licence requirements.

4.1.2 Summary of licence conditions to limit and control the release

270. A number of licence conditions have been imposed to limit and control the proposed release based on the above considerations. These include requirements to:

- limit the release to a maximum of 1000 trial participants inoculated with the GM vaccine at designated clinical facilities
- restrict exposure of at-risk individuals by specific exclusion criteria
- restrict trial participation to healthy individuals who do not have current or recent history (past 5 years) of gastrointestinal disorders including Cholera infection
- instruct the trial participants and carers/parents to practice good hygiene
- restrict the method of administration of the GM vaccine to oral ingestion of the vaccine suspension
- ensure that vaccination be performed by trained nurses and/or physicians at clinical facilities in accordance with standard universal precautions and ICH-GCP, and that appropriate personal protective equipment is worn
- store and transport all GM vaccines in accordance with relevant regulations and guidelines
- dispose of all waste in accordance with standard clinical waste disposal practices.

271. The licence holder is also required to comply with TGA requirements for the conduct of clinical trials.

4.1.3 Measures to control other activities associated with the trial

272. The Regulator has issued *Guidelines for the Transport, Storage and Disposal of GMOs* (http://www.ogtr.gov.au/). Licence conditions based on these guidelines and policies have been included regarding transportation, storage and disposal of the GMO.

273. Conditions applying to the collection of samples for experimental analyses are also included in the licence.

4.2 Other risk management considerations

274. All DIR licences issued by the Regulator contain a number of general conditions that relate to general risk management. These include, for example:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting structures, including a requirement to inform the Regulator if the applicant becomes aware of any additional information about risks to the health and safety of people or the environment

- a requirement that the applicant allows access to the trial sites by the Regulator, or persons authorised by the Regulator, for the purpose of monitoring or auditing.

4.2.1 Applicant suitability

275. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under section 58 of the Act matters that the Regulator must take into account include:

- any relevant convictions of the applicant (both individuals and the body corporate)
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

276. The Regulator considered the suitability of the applicant when the application was received. The Regulator reassessed suitability of PaxVax, including their arrangements with contractors, before making the decision to issue a licence for this application (DIR 126).

277. The licence conditions include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

278. In addition, any applicant organisation must have access to a properly constituted Institutional Biosafety Committee and be an accredited organisation under the Act.

4.2.2 Contingency plan

279. PaxVax is required to submit a contingency plan to the Regulator prior to conducting any dealings authorised by the licence. This plan will detail measures to be undertaken in the event of any unintended presence of the GM vaccines outside of the permitted areas.

280. PaxVax is also required to provide a method to the Regulator for the reliable detection of the presence of the GMOs and the introduced genetic materials in a recipient organism. This instrument will be required prior to conducting any dealings authorised by the licence.

4.2.3 Identification of the persons or classes of persons covered by the licence

281. The persons covered by the licence are the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to commencing the clinical trial, PaxVax is also required to provide a list of people and organisations who will be covered, or the function or position where names are not known at the time.

4.2.4 Reporting requirements

282. The licence obliges the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the trial
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the trial.

283. The licence holder will also be obliged to submit an Annual Report within 90 days of the anniversary of the licence containing any information required by the licence, including the results of inspection activities.

4.2.5 Monitoring for Compliance

284. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

285. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

286. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to health and safety of people or the environment could result.

Section 5 Conclusions of the consultation RARMP

287. The risk assessment concludes that this proposed limited and controlled release of the GM vaccine to take place in clinical facilities in QLD, SA, VIC and WA, involving up to 1000 trial participants and expected to run for approximately one year (depending upon the enrolments), poses negligible risks to the health and safety of people or the environment as a result of gene technology.

288. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. Licence conditions have been imposed to limit the release to the size, locations and duration requested by the applicant, and to require controls to minimise unintended exposure to the GM vaccine, as these were important considerations in establishing the context for assessing the risks.

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Appendix A. Summary of submissions from prescribed experts, agencies and authorities

The Regulator received several submissions from prescribed experts, agencies and authorities[7] on the consultation RARMP. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of the currently available scientific evidence and were used in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence. Advice received is summarised below.

View (general tone): n = neutral; x = do not support; y = support

Abbreviations: EX: Exposure; HGT: Horizontal gene transfer; HREC: Human Research Ethics Committee; LC: Licence conditions R: Risk Assessment and Risk Management Plan (RARMP) ST: Septic tank; TGA: Therapeutic Goods Administration; UE: Unintended effects; WHO: World Health Organisation

Sub. No:	View	Issue	Summary of issues raised	Comment
1	N	-	Agrees with the overall conclusion of the RARMP.	Noted
		EX	The Regulator should further consider potential exposure of contacts less than 2 years of age.	The RARMP has been updated to consider likelihood of exposure of children and their household contacts.
		LC	The Regulator should further consider other possible exclusions relating to other vaccinations.	The RARMP has been updated to include discussion of the WHO recommendation regarding simultaneous vaccination with live typhoid vaccine and the potential adverse effects. This information will also be forwarded to the TGA, and the applicant will be advised that they should inform the HRECs overseeing the trial of this information.
		R	The Regulator should consider more specificity related to the method of managing batch variation.	The information relating to the method of managing batch variation was considered by the Regulator. However, this information has been requested to be considered as Confidential Commercial Information (CCI) under section 185 of the Act and so is not included in the RARMP
2	N	-	Has no comment to put forth in relation to the proposal.	Noted

[7] Prescribed agencies include GTTAC, State and Territory Governments, relevant local governments, Australian Government agencies and the Minister for the Environment.

Sub. No:	View	Issue	Summary of issues raised	Comment
3	Y	HGT	Overall finds no real risk with the vaccine and support the proposed trail. Suggests that consideration should be given to HGT and recombination events as they could lead to reversion of vaccine strain to pathogenic form.	The potential of unintended changes in bacterial characteristics due to horizontal gene transfer was evaluated in detail. The RARMP concludes that this is not likely to occur and the risk is negligible.
		LC	Queries what limits and controls are in place for GM vaccine after patient leaves the clinical facility. Acknowledges that there are conditions around handling and disposal of GMOs.	GM cholera vaccine without the bicarbonate buffer is very susceptible to hot and dry environmental conditions (needs refrigeration for storage) and available scientific evidence shows that the GM bacteria does not survive very long in soil.
4	Y	-	Satisfied with the conclusion of the draft RARMP	Noted
5	Y	-	Supports the overall conclusions of the RARMP	Noted
6	Y	-	Supports the overall conclusions of the RARMP	Noted
7	Y	-	Supports the overall conclusions of the RARMP and believes that the proposed trial does not pose any risk to the public or to the environment.	Noted
8	N	-	Do not have any comments to make on this application as it is has no food related impact.	Noted.
9	Y	ST	Supports overall conclusions of the RARMP. Suggests considering likelihood of GM bacteria escaping the septic tanks as some trail participants may be using them.	Noted The RARMP has been updated to consider likelihood of GM bacteria escaping the septic tank. This did not impact the risk assessment for the associated risk scenarios. The risks are still considered negligible.
10	Y	-	Have no concerns with the proposed trial given the conditions specified in the RARMP.	Noted

Appendix B. Summary of submissions from the public

The Regulator received 68 submissions from the public on the consultation RARMP. The issues raised in this submission are summarised in the table below. All issues raised in submissions that related to risks to the health and safety of people and the environment were considered in the context of currently available scientific evidence in finalising the RARMP that formed the basis of the Regulator's decision to issue the licence.

Type: All submissions were received from individual members of the public and opposed the trial.

Issues raised: A: Why in Australia; **CR**: Confidentiality request; **Ef**: Efficacy; **EP**: Environmental persistence; **HCO**: Haitian cholera outbreak; **HTP**: Health of trial participants; **HGT:** Horizontal gene transfer; **I:** Immunogenicity data; **NC**: Non-compliance; **O**: Orochol®vaccine; **R/B**: Risk/Benefit analysis; **T:** Transmission; **TD**: Trial design; **UE**: Unintended effects; **V**: Vaccination.

Other abbreviations: **Act**: *Gene Technology Act 2000*; **GM**: Genetically modified; **GMO**: Genetically modified organism; **TGA**: Therapeutic Goods Administration; **USA**: United States of America; **WHO:** World Health Organisation

Sub. No:	Issue	Summary of issues raised	Comment
1-68	-	Opposes clinical trial of GM cholera vaccine.	The Regulator makes licence decisions on the basis of consideration of risks to people and the environment. The RARMP concluded that risks to human health and safety as a result of this clinical trial are negligible.
8, 16	V	Opposes vaccination in general.	The Regulator makes licence decisions on the basis of consideration of risks to people and the environment. The RARMP concluded that risks to human health and safety as a result of this clinical trial are negligible.
2-5, 10, 12, 13, 15-58, 60-68	A	Considers that risks associated with this clinical trial are not justifiable because cholera is a very rare disease in Australia (4-5 cases per year). Queries why trials are not taking place in areas with endemic cholera, or in the US where the vaccine is manufactured.	The vaccine is being developed as a voluntary vaccine for people travelling to areas where cholera is an endemic disease, and may be used in developing countries in response to cholera outbreaks. In areas where cholera is endemic, people are likely to have already been exposed to the disease and already have some level of immune response. People in Australia and other locations where the trial will take place (USA, Canada) are less likely to have been exposed to the cholera bacteria, and therefore less likely to have an existing immune response against the bacteria. Performing the clinical trial in non-endemic areas will lead to much clearer results.
5, 8, 10,17, 19-58, 60-67	HGT	Concerned by potential for gene transfer from GM cholera vaccine to other bacteria in the environment.	The potential for harm to people and the environment due to horizontal gene transfer was evaluated in detail. The RARMP concludes that this is not likely to occur and the risk is negligible.

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5,15, 19-67	NC, T	Considers that insufficient data was provided by the applicant to support the claim that the GM bacteria are destroyed by stomach acid. Concerned that trial participants (especially children) will not fully comply with hygiene measures, leading to infection of people outside the trial with the GM cholera vaccine.	The GM bacteria is not able to cause disease. Vaccination requires a dose of 8 x 10^8 CFU given in a buffer solution to survive stomach acid. After vaccination, less than one third of clinical trial participants may shed a small amount (10^4) of the GM bacteria for about a week. This is much less than the vaccine dose and it is highly unlikely that such a small number of bacteria consumed without buffering would be able to survive in the stomach. In previous clinical trials, where transmission of vaccine strain to close contacts / family members of trial participants was investigated, either no transmission was detected or very low level of transmission was reported (1 of 177 unvaccinated family contacts). The RARMP has been updated and the likelihood of transmission of GM bacteria from children participating in the trial has been further evaluated. The RARMP concludes that transmission is an unlikely event which poses negligible risks to people and to the environment.
5, 19-67	EP	Concerned about disposal of GM cholera vaccine into sewage or dump sites (via nappies), and the potential for: • exposure of people not participating in the trial that have not agreed to be exposed to the GM cholera vaccine; • adverse health effects or cholera outbreaks; and • persistence of GM vaccine in water supplies due to the Mercury resistance gene.	GM cholera vaccine is not able to cause disease. Without the bicarbonate buffer it is very susceptible to hot and dry environmental conditions. GM cholera vaccine was not found in the sewerage from houses of people involved in previous clinical trials. In Australia, treatment of wastewater (including sewage) is required as per State and Territory regulations. The GM bacteria is unlikely to spread and persist following wastewater treatment. As discussed in Chapter 2, Section 2.2 of the RARMP, explicit instructions will be given for nappy disposal, further limiting the likelihood of GM bacteria entering into the environment. Additionally, available scientific evidence shows that the GM bacteria do not survive very long in soil.
3- 6, 10, 14-16, 51, 59	HTP, TD	Expressed concerns over various aspects of the clinical trial design including: • the ethics of child participation and consent; • requirement for independent oversight of trial participant's safety; • long term follow up of participant's health; • provisions for compensation for any post-vaccination complications; • the nature of information provided to trial participants; and • whether the trial as currently designed would produce meaningful data.	Design of clinical trials is beyond the scope of the Act. The trial also needs to meet requirements of the Therapeutic Goods Administration, which is responsible for regulation of medicines in Australia, and to comply with Australian human research ethics standards. Approval by a Human Research Ethics Committee (HREC) at each trial site is a fundamental requirement of any clinical trial. HRECs conduct both ethical and scientific assessment of the proposal and in addition often consider issues of research governance. Other elements of governance of clinical trials that are considered by HRECs include appropriate informed consent, specific inclusion and exclusion criteria, data monitoring and vaccine accounting and reconciliation.

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4-6, 8-10, 12, 24, 63	O, UE	Considers that data should be provided as to how prevalent this vaccine currently is in the environment following its previous release, and what environmental harms it may have caused. Considers that insufficient data was provided by the applicant regarding removal of the Orochol®vaccine from the market, to demonstrate that the removal was not risk related. Considers that understanding of GMOs is limited and therefore the application involves unpredictable risks, particularly in the long term. There could be unforeseen adverse effects on human health or unexpected interactions with other bacteria.	Prior to being registered for use as a prescription medicine, the TGA evaluation concluded that Orochol® was "of acceptable standard of quality, safety and efficacy for registration as a prescription medicine for the proposed human use". Commercial release of Orochol® was previously approved by the Gene Technology Regulator under licence DIR 033. The RARMP for DIR 033 concluded that Orochol® vaccine poses no risks to public health and safety or to the Australian environment that require managing through a risk management plan. Orochol® was also sold in 17 countries other than Australia, with over 500,000 doses distributed worldwide and had no significant safety concerns raised or significant adverse events reported. Additionally, there are no reports of environmental harm attributable to the previous commercial release. Orochol® is no longer available as the manufacturer stopped production of the vaccine voluntarily in 2004 and voluntarily surrendered approvals due to business considerations. Consistent with DIR 033, the current RARMP prepared for DIR 126 concludes that the current clinical trial poses negligible risks to human health and safety and the environment.
5,6	I, Ef	Considers that immunogenicity data from previous trials should be presented in the RARMP and states that the vaccine has not been shown to provide effective protection against challenge with wild-type cholera. According to a WHO report, Orochol® vaccine at the dose proposed for the Australian clinical trial failed to demonstrate protection during a study in Indonesia, where cholera is endemic. Therefore, Australian trial participants may not be protected against contracting cholera.	The RARMP has been updated to include the immunogenicity data from previous trials and to further elaborate on the study conducted in Indonesia. The purpose of this trial is to verify the effectiveness of the GM vaccine for protecting people travelling to cholera endemic areas against cholera infection. Therefore, the results from this trial would inform any future applications for commercial release as a travel vaccine. The trial also needs to meet requirements of the Therapeutic Goods Administration, which is responsible for the evaluation and approval of human medicines in Australia. Efficacy of human vaccines is considered by the TGA.
5	HCO	Recommends that the RARMP consider claims that the recent Haitian cholera outbreak was caused by distribution of live recombinant cholera vaccine by aid workers.	The RARMP has been updated with further discussion of the Haitian cholera outbreak, and the most likely cause. There is no evidence that the vaccine proposed to be used for the current trial or any other genetically modified cholera vaccine was involved in the Haitian cholera outbreak.
5	R/B	Considers that the RARMP should include a risk/benefit analysis.	Under the legislation, benefits of gene technology are outside the matters to which the Regulator may have regard when deciding whether or not to issue a licence. The RARMP concluded that risks to human health and safety and the environment are negligible.
15	CR	Considers that there are no grounds for keeping information on the GMO confidential and that this information should be released to independent scientists and the public.	The application for Confidential Commercial Information (CCI) received from PaxVax was considered in accordance with the requirements of the Act. Information that could reveal CCI has not been included in the publicly released RARMP. CCI is available to the OGTR, prescribed experts and Commonwealth and State government agencies for the purposes of risk assessment.