Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 174

Commercial supply of a genetically modified cholera vaccine, Vaxchora®

Applicant: Biocelect Pty Ltd

February 2021

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application DIR 174

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence application (DIR 174) for import, transport, storage and disposal of a genetically modified (GM) cholera vaccine, Vaxchora®, for the purpose of its commercial supply as a human vaccine.

Before the GM vaccine can be used as a human vaccine, Biocelect must also obtain regulatory approval from the Therapeutic Goods Administration (TGA). Therapeutic goods for sale in Australia must be included in the Australian Register of Therapeutic Goods (ARTG) under the *Therapeutic Goods Act 1989*. The TGA would assess patient safety, quality and efficacy prior to including the GM vaccine on the ARTG. In addition, approval from the Department of Agriculture, Water and the Environment will also be required for import of the GM vaccine.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed supply of the GM cholera vaccine poses negligible risks to human health and safety and the environment and no specific risk treatment measures are imposed. However, general licence conditions have been imposed to ensure that there is ongoing oversight of the release.

The application

Application number	DIR 174
Applicant	Biocelect Pty Ltd (Biocelect)
Project title	Commercial supply of a genetically modified cholera vaccine, Vaxchora®[1]
Parent organism	*Vibrio cholerae* strain 569B
Introduced gene and modified trait	• 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 - to allow identification of GM strain)
Previous releases	Commercial supply of the GM *V. cholerae* strain as a human vaccine (formerly known as Orochol®) was previously approved in Australia. The GMO described in this application is the same GM *V. cholerae* strain as previously approved GM cholera vaccine Orochol®. <u>Orochol®:</u> • Commercial supply of the GM cholera vaccine, Orochol®, as a human vaccine was previously approved by the Genetic Manipulation Advisory Committee (GMAC), the Therapeutic Goods Administration (TGA) and subsequently by the Gene Technology Regulator under DIR 033. The licence

[1] The title of the licence application submitted by Biocelect is "*Commercial use of Vaxchora® for immunisation against cholera*".

	for DIR 033 was issued to CSL Ltd on 20 June 2003 and was surrendered at the licence holder's request on 14 September 2010. • Orochol® was previously registered for commercial sale in several other countries including Switzerland, Austria, Finland, Canada, New Zealand, Sri Lanka, the Philippines and several South American countries. <u>Vaxchora®:</u> • Clinical trials with PXVX0200 (trade name Vaxchora®) were approved and conducted in the United States (US) to study the safety and effectiveness of the vaccine in preventing cholera. • Clinical trials (limited and controlled release) of this GMO as a human vaccine (PXVX0200, trade name Vaxchora®) were approved by the Gene Technology Regulator under DIR 126. This was to confirm the safety and efficacy of the newly manufactured product. The licence for DIR 126 was issued to PaxVax Australia Pty Ltd on 10 April 2014 and was surrendered at the licence holder's request on 10 September 2020.
Current approvals	• Vaxchora® has been approved for oral administration by the Food and Drug Administration (FDA) for adults and by the European Medicines Agency (EMA) for adults and children aged 6 years and older traveling to cholera-affected areas.
Proposed locations	Australia-wide for travellers
Primary purpose	Commercial supply of the GM cholera vaccine

Risk assessment

The risk assessment concludes that risks to the health and safety of people or the environment from the proposed dealings, either in the short or long term, are negligible. No specific risk treatment measures are required to manage these negligible risks.

The current assessment focuses on risks posed to people (other than the intended vaccine recipient) and to the environment, including long term persistence of the GMOs, which may arise from the import, transport, storage or disposal of the GMO. The risk assessment process considers how the genetic modification and activities conducted with the GMO might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application, relevant previous approvals, current scientific knowledge and advice received from a wide range of experts, agencies and authorities consulted on the preparation of the RARMP. Both the short and long term risks were considered.

Credible pathways to potential harm that were considered included: whether people and animals can be inadvertently exposed to the GMO, the potential for the reversion of GMO to the toxigenic phenotype and the potential for transfer of genetic material to and from the GMO. The potential for GMO to be released into the environment and its effects was also considered.

The principal reasons for the conclusion of negligible risks are that: the genetic modifications make the GMO unable to cause disease therefore are unlikely to cause harm to people or the environment; genes similar to the introduced genes are present in the environment; *V. cholerae* does not cause disease in other organisms; likelihood of reversion of GMO to a toxigenic strain is very low and the impact of persistence of the small numbers of GMO in the Australian aquatic environment is negligible.

Risk management

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 identified risks and considers general risk management measures. The risk management plan is given effect through licence conditions.

The risk management plan concludes that risks from the proposed activities can be managed so as to protect people and the environment by imposing general conditions to ensure that there is ongoing oversight of the release.

As the level of risk was assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions regarding post-release review (PRR) to ensure that there is ongoing oversight of the supply of the GM cholera vaccine and to allow the collection of information to verify the findings of the RARMP. The licence also contains a number of general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements, which include an obligation to report any unintended effects from activities with the vaccine.

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Abbreviations

AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
ARTG	Australian Register of Therapeutic Goods
CFU	Colony forming units
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
DAWE	Department of Agriculture, Water and the Environment
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EU	European Union
FSANZ	Food Standards Australia New Zealand
g	gram
GM	Genetically modified
GM bacteria	*V. cholerae* CVD 103-HgR
GMAC	Genetic Manipulation Advisory Committee
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal gene transfer
hlyA	Haemolysin gene
IATA	International Air Transport Association
kb	Kilobase pair of DNA
LGA	Local government area
Mb	Mega base pairs
mer	Mercury resistance genes/operon from *Shigella flexneri*
min	minute
ml	Milli litre
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
PCR	Polymerase chain reaction
QLD	Queensland
RARMP	Risk Assessment and Risk Management Plan
S. flexneri	*Shigella flexneri* (bacterial species that causes bacillary dysentery)
TCP	Toxin co-regulated pili

tcp	Toxin co-regulated pili gene
TGA	Therapeutic Goods Administration
the Act	The *Gene Technology Act 2000*
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
USA	United States of America
V. cholerae	*Vibrio cholerae* (bacterial species that causes cholera)
WA	Western Australia
WHO	World Health Organization

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 and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, 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. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The *Risk Analysis Framework* (RAF) (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR) website.

5. Figure 1 shows the information that is considered, within the regulatory framework above, in establishing the risk assessment context. This information is specific for each application. Potential risks to the health and safety of people or the environment posed by the proposed release are assessed within this context. Chapter 1 provides the specific information for establishing the risk assessment context for this application.

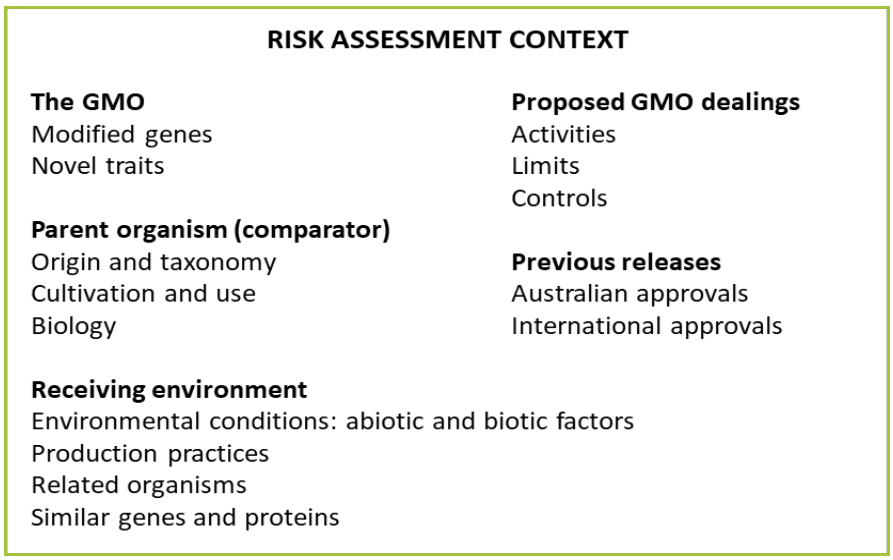

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. Since this application is for commercial purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. Therefore, under section 50(3) of the Act, the Regulator was required to seek advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the RARMP. This first round of consultation included the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government

authorities or agencies prescribed in the Regulations, all Australian local councils[2] and the Minister for the Environment. A summary of issues contained in submissions received is provided in Appendix A.

7. Under Section 52 of the Act the Regulator was required to conduct a second round of consultation, to seek comment on the RARMP from the Gene Technology Technical Advisory Committee (GTTAC), State and Territory Governments, Australian Government authorities or agencies prescribed in the Regulations, Australian local councils and the Minister for the Environment, as well as the public. A summary of the advice from the prescribed experts, agencies and authorities in the second round of consultation, and how it was taken into account, is presented in Appendix B. Two public submissions were received and their consideration is summarised in Appendix C.

1.1 Interface with other regulatory schemes

8. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Water and the Environment (DAWE).

9. To avoid duplication of regulatory oversight, risks that have been considered by other regulatory agencies would not be re-assessed by the Regulator.

10. For the commercial supply of a live GM vaccine, dealings regulated under the Act include the import, transport and disposal of GMOs. The Regulator has assessed risks to people as a consequence of conducting these activities and risks from persistence of the GMOs in the environment.

11. The DAWE regulates products imported into Australia to protect Australia from biosecurity risks. Under the *Biosecurity Act 2015*, the importation of biological material such as live GM vaccines requires a permit from DAWE.

12. The TGA provides a national system of controls for therapeutic goods. It administers the provisions of the *Therapeutic Goods Act 1989* which specifies the standard that must be met before a vaccine can be registered on the Australian Register of Therapeutic Goods (ARTG). Inclusion in ARTG is required before a vaccine can be lawfully supplied in Australia. As part of this process, the TGA would assess the quality, safety and efficacy of the vaccine. Quality aspects could include batch-to-batch consistency in vaccine composition, purity and potency. Safety aspects could include toxicological and allergenicity profile of the vaccine, including any excipients, by-products and impurities from manufacture.

13. The administration/use of GMOs as therapeutics is not regulated under gene technology legislation. The Regulator notes that as part of the safety assessment, the TGA would evaluate viral shedding as well as risks to vaccine administrators, recipients and their carers who may be present during administration of a vaccine. The Regulator does not assess vaccine excipients and would not assess manufacturing by-products and impurities unless they are GM products.

14. The labelling, handling, sale and supply of scheduled medicines is regulated through the *Scheduling Policy Framework for Medicines and Chemicals* (AHMAC, 2018). Guidelines for the safe handling, storage and distribution of Schedule 4 medicines such as vaccines are specified through the *Australian Code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG, 2011). The

[2] Biocelect is seeking approval for unrestricted commercial supply of the GM cholera vaccine in Australia. Therefore, the Regulator decided to consult with all of the local councils in Australia, except for those that have requested not to be consulted on such matters.

provisions of this Code, which ensure that quality is maintained during wholesaling, are applied through applicable State and Territory therapeutic goods/drugs and poisons legislation, and/or State or Territory wholesaler licensing arrangements.

Section 2 The proposed dealings

15. Biocelect Pty Ltd (Biocelect) proposes the commercial supply of a genetically modified (GM) cholera vaccine, Vaxchora® to occur Australia-wide, subject to any moratoria imposed by States and Territories for marketing purposes. Vaxchora® has been developed as a travel vaccine, for active immunisation against cholera disease caused by *Vibrio cholerae* serogroup O1 in adults and children aged 2 years and older who would be visiting cholera-affected countries.

16. If approved by both the Regulator and the TGA, Biocelect intends to supply Vaxchora® to medical facilities such as specialist travel clinics, pharmacies and general practitioners and to surgical/medical wholesalers. Vaxchora® would be made available as a Schedule 4 prescription medicine and would be self-administered either at medical facilities or at home.

17. For the ongoing commercial supply of Vaxchora®, the dealings assessed by the Regulator are:

(a) import the GMO

(b) transport the GMO

(c) dispose of the GMO

and the possession (including storage), supply or use of the GMO for the purposes of, or in the course of, any of the above.

2.1 Details of the proposed dealings

18. Vaxchora® contains one active ingredient sachet and one buffer sachet. The active ingredient is packaged as 2 g of lyophilised (freeze-dried) powder for oral suspension containing 4×10^8 to 2×10^9 colony forming unit (CFU) of GMO i.e., *V. cholerae* CVD 103-HgR in single-use sachet made from four-ply multilayer foil. In addition, a buffer sachet containing 4.5 g of effervescent powder intended to temporarily neutralise stomach acids is packed in a three-ply multilayer foil sachet. The active ingredient sachet and buffer sachet are then packed in a cardboard carton. The carton is labelled to contain a statement about a GMO i.e., “This medicine contains genetically modified organisms. Unused medicine must be disposed of in compliance with the local biosafety guidelines”.

19. The preparation of the vaccine would vary depending on whether administration would be to children or adults.

- For adults and children aged 6 years and older, the vaccine would be prepared by adding the contents of the buffer sachet into a cup containing 100 ml of water followed by addition of the contents of an active ingredient sachet.
- For children aged between 2 and 6 years, the vaccine would be prepared by adding the contents of a buffer sachet into a cup containing 100 ml water followed by discarding half (approximately 50 ml) of the buffer solution. The active ingredient sachet is then added to the buffer solution. The reduction in buffer concentration would 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.

20. Vaxchora® would be imported from Emergent BioSolutions, Berne, Switzerland. The import requires a permit from the DAWE and authorisation from the TGA.

21. Vaxchora® would be first imported into the DHL’s HP8 cold chain facility. Vaxchora® would be then distributed by DHL or another commercial courier/delivery service to medical facilities including surgical/medical wholesalers and TGA (if required).

22. Transport of Vaxchora® will follow the *Guidelines for the Transport, Storage and Disposal of GMOs* and/or International Air Transport Association (IATA) requirements. Vaxchora® would be transported and stored at 2-8°C according to the National Vaccine Storage Guidelines (Department of Health, 2019) and the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP, 2020).

23. Vaxchora® is intended to be administered at medical facilities (i.e. pharmacy, travel clinic or GP surgery). However, in certain circumstances such as when the vaccine recipient has consumed food or drink 60 minutes before coming to the medical facility, Vaxchora® would be prepared and administered in the home.

24. Disposal of the active ingredient sachet and single-use items (cup and spoon) as well as unused/expired vaccine at the medical facilities will be discarded into pathological waste. This waste would be then decontaminated by a method approved by the Environmental Protection Agency or Health Department of each State/Territory. If vaccine is taken at home, the empty vaccine sachet and single-use items (cup and spoon) will be disposed of in household waste and will ultimately be carried to land fill. When non-disposable items are used with the vaccine at home, users will be instructed to wash the items in hot soapy water or in a dishwasher to kill the GMO.

Section 3 Parent organism

25. The parent organism is the human bacterium *Vibrio cholerae* strain 569B. The bacteria belongs to the genus *Vibrio* in the *Vibrionaceae* family and is classified as a Risk Group 2 organism (Standards Australia/New Zealand, 2010). The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of *V. cholerae* will be discussed here.

26. The parent organism *V. cholerae* strain 569B was first isolated from a patient in 1948 in India (Bik et al., 1996). In comparison to other toxigenic *V. cholerae* strains, *V. cholerae* strain 569B is considered to be a weak pathogen as it does not cause diarrhoea because it lacks one of the common toxins i.e., Shiga-like toxin. It also unable to produce a functional haemolysin protein due to a naturally arising mutation in the haemolysin (*hlyA*) gene and is therefore unable to cause break down of red blood cells (Alm et al., 1988). This strain is also a non-permissive host[3] for bacteriophages. However, it is still regarded as a toxigenic strain because it does produce classical cholera toxin and causes mild cholera disease symptoms (Alm et al., 1988).

3.1 Basic biology of *V. cholerae*

27. Cholera is an infectious disease caused by infection with *V. cholerae* bacteria. Cholera is typically acquired following ingestion of food or water contaminated with *V. cholerae* and causes rapidly dehydrating diarrhoea which can range from mild to life threatening.

28. *V. cholerae* is a gram negative, comma shaped bacteria which is non-invasive[4] and highly motile. This bacteria can survive in the presence of oxygen as well as in the absence of oxygen such as inside the human gastrointestinal tract.

29. *V. cholerae* is classified into more than 200 serogroups based on the O antigens on the cell surface. Some serogroups cause cholera disease in humans (toxigenic; toxin producing bacteria) while others are not associated with the cholera disease (non-toxigenic serogroups).

[3] Non-permissive host is an organism that does not support growth or replication of bacteriophages and is resistant to bacteriophage infection.

[4] Non-invasive bacteria are those which colonise the small intestine but do not invade the cells.

30. Only serogroup O1 and serogroup O139 are associated with severe cholera disease. However, some serogroups of *V. cholerae* (non-O1 and non-O139) can cause bloody diarrhoea, gastroenteritis and extra-intestinal infections but do not cause cholera.

31. The two major virulence factors associated with the cholera causing *V. cholerae* strains are cholera toxin (CTX) and toxin co-regulated pilus (TCP) (Almagro-Moreno and Taylor, 2013). These virulence factors are encoded within mobile genetic elements, CTX is encoded within the filamentous phage CTXϕ and TCP within Vibrio pathogenicity island-1 (Sakib et al., 2018).

32. CTX is a member of the A-B enterotoxin family and is made up of enzymatically active A polypeptide (*ctxA*) and receptor-binding B (*ctxB*) subunits. CTXA is the cause of the watery diarrhoea.

33. TCP is a self-binding pilus that binds bacterial cells together and allows the bacteria to become established in the human small intestine. This feature has led to TCPs being described as adhesion factors or colonisation factors. TCPs are very specific to human intestine cells. Therefore, TCPs play a major role in restricting the host range of toxigenic *V. cholerae* to humans (Kaper et al., 1995).

34. The *V. cholerae* O1 serogroup consists of two distinct biotypes, Classical and El Tor. These are designated based on their physiological properties including polymyxin B resistance, number of CTX-encoding genes, haemolysin activity and the presence of the mannose-sensitive hemagglutinin (Reidl and Klose, 2002; Nelson et al., 2009). These biotypes are further divided into two serotypes, Ogawa and Inaba, which differ by a single methyl group in the terminal sugar of the O-antigen polysaccharide (Harris, 2018).

35. The *V. cholerae* genome consists of two circular chromosomes. Chromosome 1 (~3Mb) is bigger than chromosome 2 (~1Mb) (Heidelberg et al., 2000). Both chromosomes are crucial as they each carry essential genes. Most of the genes required for growth and viability are located on chromosome 1, although some genes essential for normal cell function are found only on chromosome 2 (Trucksis et al., 1998; Heidelberg et al., 2000).

3.2 The cholera toxin and disease

36. *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 CTX is a well characterised 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 which is responsible for the biological activity of the toxin (diarrhoea) and the helically structured A2 fragment forms a link to the five identical B subunits (Figure 2). The A2 and B subunits are non-toxic and help cause disease by binding the toxin to receptors on intestinal membranes which stimulates an immune response.

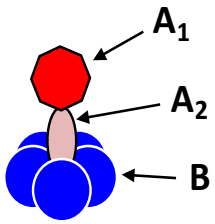

Figure 2 Structure of cholera toxin

37. Once inside the cell, the A1 fragment binds to G proteins which are involved in transmitting signals / stimuli to the cells. This induces a cascade of events in the epithelial cells, including 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 which is characteristic of *V. cholerae* infection (Spangler, 1992; Mekalanos et al., 1997).

38. Although CTX is responsible for the profuse watery diarrhoea typical of cholera, there are other proteins expressed by *V. cholerae* O1 which contribute to the severity of symptoms. Some of the well characterised proteins are: Zot (zonula occludens toxin), which can increase the permeability of the small intestinal mucosa; Ace (sodium channel inhibitor), which can cause fluid accumulation; haemolysin/cytolysin, which can break open a variety of cells, and Shiga-like toxin, which is known to cause similar effects as CTX (Kaper et al., 1995).

39. *V. cholerae* is ingested following consumption of contaminated food or water. However, the majority of bacteria are killed by gastric acid in the stomach as *V. cholerae* is sensitive to low pH. For the onset of severe cholera in an otherwise healthy person, a high infectious dose of more than a million ($\sim 10^8$ CFU) 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). A few of the surviving bacteria penetrate the lining of the intestine and colonise the small intestine. These bacteria eventually produce CTX in the small intestine which results in the development of clinical symptoms of cholera (Reidl and Klose, 2002; Nelson et al., 2009; Harris, 2018).

40. Approximately 5-10% of infected people exhibit severe disease which is characterized by profuse watery diarrhoea, vomiting, and leg cramps. During the acute phase of cholera, up to half the body weight can be lost in 24 hours. 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.

41. 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 after infection. However, if patients are sufficiently rehydrated during the acute phase of the disease, survival rate is nearly 100%.

42. As mentioned above, the parent organism (*V. cholerae* strain 569B) has reduced pathogenicity compared to other toxigenic strains of *V. cholerae* serogroup O1.

3.3 Epidemiology

3.3.1 Pattern of distribution

43. Cholera is endemic in areas with poor infrastructure and sanitation such as parts of Asia, Africa, and Latin America. Cholera also occurs sporadically or as limited outbreaks in countries due to flooding following natural disasters such as earthquakes and cyclones.

44. There have been seven main cholera pandemics that 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 classical strains are believed to be responsible for the six previous cholera pandemics whereas El Tor is responsible for the seventh cholera pandemic (Kitaoka et al., 2011; Harris et al., 2012; Hu et al., 2016).

45. WHO estimates that 1.3–4 million cases of cholera occur per year, killing 21 000 to 143 000 people annually, predominantly in Asia and Africa (Harris et al., 2012; WHO, 2019).

46. The risk of infection is estimated to be 0.2 cases per 100,000 travellers from Western countries. However, this estimate is likely to be under-detected and under-reported (Australian Immunisation Handbook, 2018).

3.3.2 Habitat and persistence of cholera in environment

47. *V. cholerae* is found as part of the normal, free living bacterial community or found associated with other aquatic organisms in environments where fresh water from rivers and streams mixes with salty ocean water particularly in tropical and subtropical regions (Harris et al., 2012; Sakib et al., 2018). The survival of *V. cholerae* in the natural environment is affected by a range of factors such as water temperature, salinity, oxygen tension, sunlight, rainfall, pH, and the availability of trace elements and

chemical nutrients (Almagro-Moreno and Taylor, 2013). These organisms grow best in the presence of slightly salty warm water that contains sufficient organic nutrients.

48. Non-O1 *V. cholerae* strains are more commonly found in the environment than O1 strains and most of the O1 strains found in the environment are CTX negative (Reidl and Klose, 2002). However, a few CTX producing *V. cholerae* strains are present in the environment (Kaper et al., 1995).

49. Upon nutrient deprivation or other environmental stressors (*temperature and salinity*), *V. cholerae* enters a dormant form called viable but not culturable (VBNC), also known as conditionally viable environmental cells (CVEC) (Kitaoka et al., 2011; Harris et al., 2012; Almagro-Moreno and Taylor, 2013). This dormant state has been described for a number of bacterial species as a survival strategy in the natural environment (Kaper et al., 1995).

50. The dormant form allows *V. cholerae* to survive in the environment by forming clumps or biofilms. Biofilms provide increased stress resistance and increased access to nutrients (Lutz et al., 2013). The clumps/biofilms of *V. cholerae* results in higher infectivity in humans due to the presence of high numbers of bacteria (Faruque et al., 2006). These biofilms attach onto living things and man-made surfaces (such as chitinous and gelatinous zooplankton and phytoplankton) and these surfaces plays a role in dispersal of *V. cholerae* throughout suitable environments (Lutz et al., 2013). The dormant VBNC form cannot be recovered by current culture techniques but are still able to cause infection and under certain conditions can revert to the culturable/infectious form.

51. Within the aquatic environment, *V. cholerae* is associated with phytoplankton, zooplankton such as copepods, crustaceans, shellfish; aquatic plants and microalgae; vertebrates including fish and waterfowl; terrestrial insects, marine birds and chironomids (Figure 3) (Reidl and Klose, 2002; Almagro-Moreno and Taylor, 2013). However, these organisms are reservoirs rather than hosts for both culturable and non-culturable *V. cholerae* (Colwell et al., 1996) because they are not infected by the bacteria. The bacteria can survive for extended periods in the copepod intestines or attached to copepod chitin shells (Lutz et al., 2013).

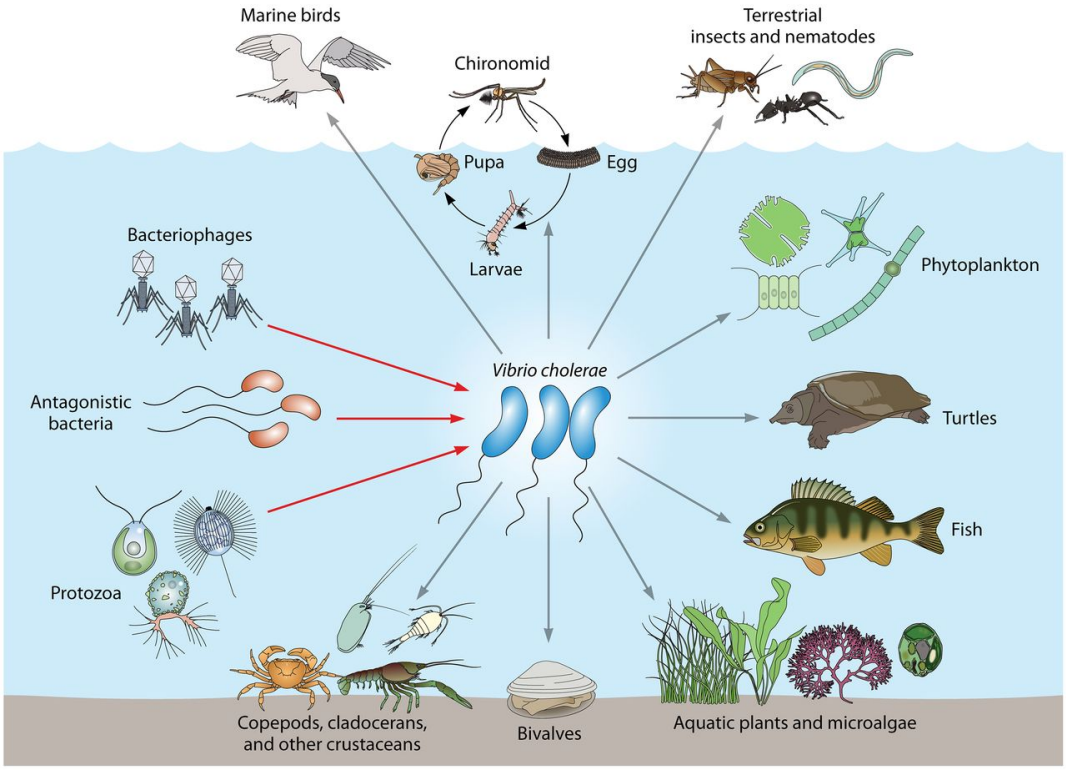

Figure 3 Vibrio cholerae interactions in its natural environment (Sakib et al., 2018). The associations of V. cholerae with reservoirs and antagonistic organisms that shape its virulence potential are shown. Grey arrows indicate reservoir and red arrows indicate antagonistic.

52. *V. cholerae* form an integral part of the native flora of aquatic environments living as free floating bacterioplanktons, attached to non-living particles or in a symbiotic relationship with a living host (Racault et al., 2019). The aquatic environment provide several advantages to the bacteria (Lutz et al., 2013; Racault et al., 2019) such as

- It provides nutrients (i.e., copepods exoskeleton can be used as a carbon source) and protection (in salinity and pH which that are detrimental to the bacteria in its free-living state) to the bacteria.
- It provides a means to transport the bacteria into new areas (e.g., with birds and insects), sometimes over long distances.
- It acts as a vector for transmission of toxigenic bacteria to humans via consumption of seafood or drinking contaminated water.
- It helps in formation of biofilms which facilitates growth, survival and persistence in aquatic environment.
- It induces the transfer and acquisition of genes by improving competency of bacterial cells (see Chapter 1, Section 3.3.5).

53. Free living *V. cholerae* have been isolated from river systems in the east and north-west of Australia, including the Rockhampton, Brisbane, Lismore, and Sydney areas (Desmarchelier et al., 1995; Siboni et al., 2016). However, cholera infections are rare due to stringent domestic water treatment, waste disposal and sewage treatment measures.

3.3.3 Host range and transmissibility

54. Humans are the only natural host of *V. cholerae*. The bacteria cannot colonise or replicate in animals and therefore are unable to cause disease in animals.

55. Humans can be exposed to *V. cholerae* due to contaminated marine waters during recreational activities (e.g., fishing) or through drinking and consumption of contaminated water or seafood respectively (Racault et al., 2019). The bacteria may be transported through long-distance oceanic corridors by currents as well as in ballast-waters from ships. In environmental water, organisms convert to a dormant form within 24 hours and these organisms are infectious upon reintroduction into humans, although the infectious dose in this form is not known. Direct transmission from person to person is uncommon (Kaper et al., 1995).

56. The incubation period for cholera can range from a few hours to five days after exposure. Symptomatic patients may shed bacteria (via faeces) 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 (Reidl and Klose, 2002; Harris et al., 2012).

57. Both CTX and TCP are essential for transmission of cholera. Ingestion of classical O395 *V. cholerae* O1 strain with deletion of *ctxA* gene, that encodes the A subunit of cholera toxin, demonstrated attenuation of cholera symptoms in human volunteers but did not impact *V. cholerae* colonisation capacity (Herrington et al., 1988). This data suggested that the *ctxA* gene is responsible for cholera symptoms and colonisation of *V. cholerae* alone did not cause the disease. In addition, ingestion of a classical O395 *V. cholerae* O1 strain with a *tcpA* gene deletion abolished the colonising capacity of the strain and subsequently cholera infection (Herrington et al., 1988; Mayo-Smith et al., 2017). This data suggested that TCP is also required for full pathogenesis of *V. cholerae* as it is required for attachment to intestinal membranes and colonisation.

58. Aerosol transmission is not reported as a disease pathway. This is possibly due to the high infectious dose of more than a million ($\sim 10^8$ CFU) live bacterial particles that are required to establish infection and cause disease.

3.3.4 Control and decontamination methods

59. The provision of safe drinking water and adequate sanitation, along with health education, food safety measures, strong disease surveillance and oral cholera vaccination are the mainstays of preventing both endemic and epidemic cholera (Centers for Disease Control and Prevention, 2018; WHO, 2019).

60. *V. cholerae* strains are sensitive to a range of antibiotics. However, strains resistant to one or more antibiotic (such as tetracycline, trimethoprim) have also been reported in both endemic and epidemic countries (Pan et al., 2008; Kitaoka et al., 2011; Centers for Disease Control and Prevention, 2018; Rijal et al., 2019; Verma et al., 2019; Das et al., 2020). The commonly used antibiotics to treat cholera are ciprofloxacin, doxycycline, and azithromycin (Mosley et al., 2017).

61. *V. cholerae* is 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 (Public Health Agency of Canada, 2010).

62. *V. cholerae* can be physically inactivated by ultraviolet light (UV; 99% inactivation at 7mJ/cm^2) and ionising radiation (>1.7 Gy X-rays) (Public Health Agency of Canada, 2010). UV light is only effective against *V. cholerae* when bacteria is present in suspension rather than embedded in particles or biofilms. In addition, *V. cholerae* does not survive well in dry conditions such as on fabric, paper, plastic and metal and dies within a few days (Felsenfeld, 1965).

3.3.5 Horizontal gene transfer

63. *V. cholerae* is a conjugative bacterium; that is, it is known to exchange genetic elements with other compatible bacteria present in the surrounding environment. Gene transfer can occur by conjugation (between strains), transformation (uptake of naked DNA) and transduction (lateral gene transfer from bacteriophages). The horizontal (to bacteria of the same or different species) and vertical (to offspring/progeny) transfer of the genes, including virulence genes by phage, pathogenicity islands and other accessory genetic elements, are responsible for helping *V. cholerae* adapt to changing environments (Heidelberg et al., 2000).

64. 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).

65. As *V. cholerae* is naturally present in coastal waters, horizontal gene transfer can be boosted by divalent cations such as Ca^{2+} and Mg^{2+} which are abundant in sea water, and other metals such as vanadium, cadmium, and nickel which are often found as pollutants in coastal systems and which improve the chances of horizontal gene transfer occurring (Racault et al., 2019).

66. Chitin and sunlight also promote horizontal gene transfer in *V. cholerae* strains by inducing natural competence for transformation and by transduction (Herrington et al., 1988; Faruque et al., 2000; Meibom et al., 2005; Udden et al., 2008; Marvig and Blokesch, 2010; Chowdhury et al., 2017; Le Roux and Blokesch, 2018). *V. cholerae* undergo transformation when they grow on chitin which enable the bacteria to take up free DNA from the environment and incorporate it into their genome. (Marvig and Blokesch, 2010; Chowdhury et al., 2017; Le Roux and Blokesch, 2018). Exposure to sunlight promotes induction of CTX prophage[5] and promotes gene transfer between V. cholerae strains (Faruque et al., 2000; Chowdhury et al., 2017).

67. Chromosomal genes are crucial for the bacteria, thus 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

[5] Prophage is the bacteriophage genetic material present in the genome of a bacterium and able to produce bacteriophages if activated.

as fertility factor, which mediates transfer of chromosomal genes via conjugation (Bhaskaran, 1959). More recently another large (80 kb) conjugative plasmid (p3iANG) was identified in some clinical and environmental strains of *V. cholerae* isolated in Africa which contain multiple antibiotic resistance genes and are able to transfer plasmids and chromosomal DNA from strain to strain (Ceccarelli et al., 2006; Valia et al., 2013).

68. *V. cholerae* strain 569B (parent organism) does not carry the P plasmid. It has one small plasmid (4.5-5 kb) with unknown function (Viret et al., 2004). When the P plasmid was experimentally introduced into *V. cholerae* strain 569B, the ability of P plasmid-containing strains to colonise the intestine was reduced five-fold in the rabbit ileal loop model (Bartowsky et al., 1990). This suggested that the presence of P plasmid in *V. cholerae* strain reduces its colonisation and therefore, transmission of cholera.

69. Bacteriophage mediated transfer of mobile genetic elements is also common in bacterial populations. A bacteriophage is a type of virus that infects and replicates in permissive 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.

70. In some strains of *V. cholerae*, part of the genome that encodes the cholera toxin (*ctxAB*) genes also includes sequences for a transmissible bacteriophage, which, if turned-on, can result in production of infectious bacteriophages. In mouse experiments, this bacteriophage has been shown to transfer the *ctxAB* genes from *ctxAB* positive strains to *ctxAB* negative strains. The bacteriophage gains entry to the bacterial cell via TCP (Waldor and Mekalanos, 1996; Mekalanos et al., 1997).

71. *V. cholerae* strain 569B (the parent organism) is a non-permissive host for this bacteriophage, making it resistant 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).

Section 4 The GM vaccine - nature and effect of the genetic modification

72. The GM vaccine consists of a live-attenuated bacterium *V. cholerae* strain CVD 103-HgR which is not able to cause cholera disease (Ketley et al., 1993). The parent strain, *V. cholerae* strain 569B, from which the vaccine strain was derived, contains two copies of the cholera toxin genes (*ctxAB*) and a single non-functional copy of the haemolysin (*hlyA)* gene on its chromosome.

4.1 The genetic modification

73. The vaccine strain was 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 (Figure 4). This was the first GMO to be registered as a live vaccine for human use in 1993 (Viret et al., 2004).

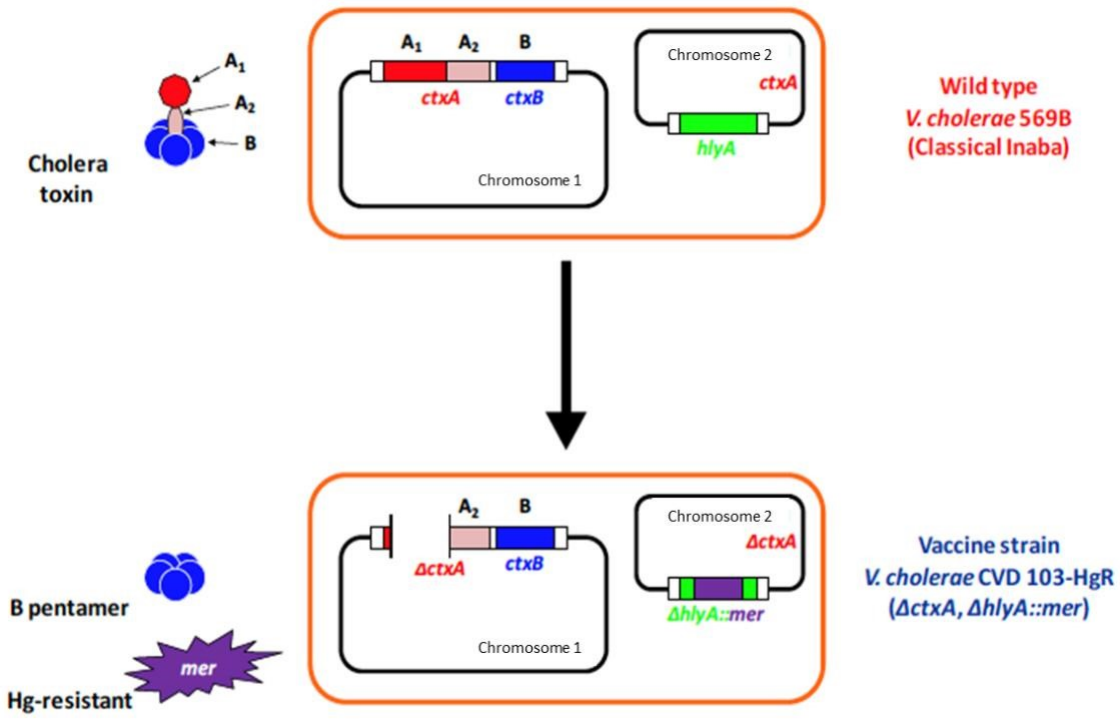

Figure 4 Construction of the GM vaccine

74. *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., 1999). The role of the *mer* operon is to protect bacteria from mercury that may be present in the environment by resisting and subsequently transforming the toxic forms of mercury to non-toxic forms. The *mer* operon is naturally spread between bacterial species (Bogdanova et al., 1998).

75. *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 and Barkay, 2012; Sone et al., 2013; Møller et al., 2014). 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). For more details please refer to Chapter 1, Section 6.3.2 of DIR-126.

76. The GM bacteria was generated through a series of 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.

4.1.1 Deletion of ctxA gene

77. The cholera toxin (ctx) subunit A and B genes are arranged on a single transcription unit (operon), within which the *ctxA* gene precedes the *ctxB* gene (Figure 4) (Mekalanos, 1983). *V. cholerae* 395 (Classical Ogawa) strain was used to isolate the cholera toxin genes.

78. Both copies of the *ctxA* gene were inactivated by deleting 550 bp of DNA and subsequently inserting the modified cholera toxin gene into *V. cholerae* 569B. The resultant strain was named *V. cholerae* CVD 103 (Kaper et al., 1984).

4.1.2 Insertion of mer operon in the hlyA gene

79. A chromosomal DNA fragment from *V. cholerae* N16961 containing the haemolysin (*hlyA*) gene was inserted where 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., 1993). The modified *hlyA* gene carrying the *mer* operon was introduced into *V. cholerae* CVD 103 via homologous

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

4.2 Effect of the genetic modification

80. Due to inactivation of both copies of the *ctxA* gene, the resultant GM strain *V. cholerae* CVD 103-HgR is not able to produce the A1 subunit of cholera toxin and therefore loses its toxic effect (Kaper et al., 1994). However, both copies of *ctxB* gene remains intact, thus the resultant strain is able to synthesise the B subunit of cholera toxin. This non-toxic B subunit is immunogenic and induces antibodies against the cholera toxin. Clinical studies of the GM vaccine show an induction of antibodies in the serum as well as other protective antibodies. These antibodies prevent the establishment and binding of bacteria in the small intestine and neutralise the cholera toxin.

81. Due to inactivation of the haemolysin A, the vaccine strain (*V. cholerae* CVD 103-HgR) is unable to produce functional haemolysin (the protein which breaks open red blood cells). Furthermore, the insertion of *mer* operon provides a selectable marker that is unique to this vaccine strain thus allowing easy and rapid differentiation between the GM bacteria and the wild-type toxigenic *V. cholerae* (Ketley et al., 1993).

82. As a result of the genetic modifications, the GM bacteria cannot produce the cholera toxin or haemolysin toxin, thus the GM bacteria does not cause cholera disease.

83. The loss of haemolysin and cholera toxin expression in the GM bacteria does not change the expression of other genes or result in the production of any novel toxic proteins or other toxic substances (Stonehouse et al., 2008). The genetic modifications will not extend the host range beyond humans as the genetic modifications are not associated with known host range determinants of *V. cholerae* (Stonehouse et al., 2008).

84. 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. In addition, the DNA and protein sequence of the *mer* operon are known and none of the *mer* operon proteins are known to have a toxic effect, or have the ability to cause a significant adverse reaction. Therefore, *mer* operon does not contribute to the disease caused by *V. cholerae* (Barrineau et al., 1984).

85. Certain members of the human gut microbiota such as lactobacilli naturally carry the *mer* operon (Osborn et al., 1997; Monachese et al., 2012). Therefore, under certain conditions (discussed below), proteins expressed by the *mer* operon can be expressed inside the human gastrointestinal tract by bacterial species naturally colonising the human gut without causing disease.

86. The *mer* operon is an inducible operon and it is activated in the presence of mercury ions. However, a low level of merR expression may occur 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 (e.g., contaminated seafood).

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

4.3 Characterisation of the GM bacteria, *V. cholerae* CVD 103-HgR

4.3.1 Genetic stability and molecular characterisation

88. The genome of the GM cholera vaccine strain present in Orochol® is fully characterised. Further, the genome of the GM cholera vaccine strain in Vaxchora® has been fully sequenced and assessed by PCR. The studies have confirmed the deletion of a substantial proportion of *ctxA* on both chromosomes

and the presence of the *mer* operon in the interrupted *hlyA* gene. Further there are no additional genomic sequences (e.g., antibiotic resistance genes) present in the genome i.e., no DNA sequences from plasmids used in construction were found to be present in the genome of the vaccine strain (Favre et al., 1996).

89. The applicant stated that sequencing analysis confirmed that the genetic changes in Vaxchora® were inherited from the parental strain CVD 103 or progenitor CVD 103-HgR. In addition, the genome sequences and microbiological characteristics of Vaxchora® and Orochol® (previously approved GM cholera vaccine) were also investigated and results showed that Vaxchora® is genetically similar to the Orochol® vaccine. Therefore, it can be concluded that no genetic modifications occurred during subsequent manufacturing of the vaccine product.

90. The genetic stability of GM cholera vaccine after prolonged storage in a lyophilised state and outgrowth for 16-17 generations and after excretion following immunisation was also assessed in a human clinical trials (Favre et al., 1996). 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 (Favre et al., 1996). 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).

91. Stability and homogeneity analysis of 16 individual colonies of Vaxchora® was assessed and all 16 colonies were found to be positive for the *mer* operon. This indicates the high stability of the chromosomally integrated *mer* operon.

4.3.2 Shedding and transmission

92. Shedding studies conducted with Orochol® vaccine (containing same GMO i.e., *V. cholerae* CVD 103-HgR strain) showed 4 x 10^4 CFU of GMO (200 GM bacteria per gram) in the faeces of 20-30% of the vaccine recipients in America (Levine et al., 1988; Cryz et al., 1992; Kotloff et al., 1992; Simanjuntak et al., 1993; Viret et al., 2004). Further, the shedding was observed in the faeces of these vaccine recipients for a maximum of 7 days with a peak on day 4 and no GMO was detected in the faeces on day 8, 11 and 15 after vaccination (Koltoff et al 1992). Similarly, GMO was detected in the faeces in 10-20% of the vaccine recipients in cholera endemic countries with a peak around day 3-4 and the GMO was not detected after 7 days (Viret et al 2004). These data suggest that Orochol® is shed by a few vaccine recipients and at low levels for a few days after vaccination.

93. Despite demonstrated shedding of live vaccine, transmission of the vaccine strain to household contacts was only observed in 1 out of 174 family contacts in a cholera-endemic country in a paediatric population (Simanjuntak et al., 1993). The data suggested that there is low frequency of transmission of vaccine strain to the household contacts (i.e., unvaccinated individuals). In addition, vaccine strain was not found in toilets or sewers near 97 households of the vaccine recipients in Indonesia (cholera endemic country) (Simanjuntak et al., 1993) suggesting that GM bacteria does not survive well in the environment. However, non-O1 *V. cholerae* (i.e., strains other than the vaccine strain) were isolated from 46 of the samples demonstrating the presence of non-toxigenic *V. cholerae* strains in these environments.

94. Similarly, shedding of Vaxchora® was evaluated in healthy adult vaccine recipients for the first 7 days post vaccination. The study showed that only 11.3% of the Vaxchora® recipients shed vaccine strain in their stool with the highest shedding on day 7 (Chen et al., 2014). The GM bacteria was not detected in either stool samples or rectal swabs collected seven days post-vaccination in any of the 24 household contacts (adults not children) of vaccine recipients. Therefore, transmission of the vaccine strain from the trial participant to their household contacts was not found (Chen et al., 2014). Furthermore, CVD 103-HgR was not recovered from toilets or sewers of households next door to vaccine recipients (Levine et al., 2017). These data suggest that Vaxchora® is shed at low levels by a few vaccine recipients and

transmission of Vaxchora® to household contacts and the presence of GM bacteria in these environment is rare. However, the maximum duration of Vaxchora® shedding is not known.

95. As the maximum duration of shedding with Vaxchora® remains unknown, data from the literature on Orochol® support the assumption that shedding will be unlikely after day 14 (Perry et al., 1998) as no vaccine shedding was seen at day 12 post immunisation. The low frequency of shedding may be related to the development of serum antibodies by most individuals, which will control infection by day 10 post vaccination.

96. Infectious dose of *V. cholerae* is greater than 10^8 CFU except in people who produce less stomach acid such as young children, the elderly and those who take antacids who may be infected with as low as 10^4 CFU (Kitaoka et al., 2011). As mentioned above based on data from Orochol®, about 10^4 CFU of GM bacteria could shed from a few vaccine recipients. The low level of shedding is unlikely to translate into an infection in healthy adults, but could be sufficient to cause infection in children and the elderly.

4.3.3 Stability in the environment and decontamination

97. Vaxchora® loses its effectiveness if left at room temperature for extended periods prior to reconstitution, therefore it needs to be stored in a refrigerator (2-8°C) and is required to be transported in refrigerated conditions.

98. The applicant stated that when high concentration of Vaxchora® was applied to stainless steel surfaces in the manufacturing facility and left at room temperature, the number of live bacteria were substantially reduced after 3 days. This suggests that Vaxchora® is unlikely to persist long term on surfaces.

99. In other studies, no live bacteria were found 14 days after inoculating non-sterile estuarine water with 2.2×10^5 CFU of the GM bacteria. Similarly no GM bacteria could be cultured from soil samples that had been inoculated with approximately 10^6 CFU of GM bacteria after 19 days (Viret et al., 2004). These studies demonstrated that GM bacteria do not multiply in soil or estuarine water (Viret et al., 2004). However, this study did not test for the presence of GM bacteria in VBNC state.

100. Methods of decontamination effective against the parent organism, *V. cholerae* strain 569B, are expected to be equally effective against the GMO (see Chapter 1, Section 3.3.4).

4.3.4 Safety and Immunogenicity

101. The GM cholera vaccine strain *V. cholerae* CVD 103-HgR confers immunity against cholera. However, safety and efficacy of Vaxchora® has not been confirmed in elderly (older than 64 years of age), pregnant women, immunocompromised subjects, and children less than 2 years of age.

102. The most common side effects following Vaxchora® vaccination are tiredness, headache, abdominal pain, nausea/vomiting and lack of appetite.

103. Extensive clinical safety data is available from the historical studies conducted with the previously marketed CVD 103-HgR Orochol®. These studies demonstrated safety and displayed significant antibody response in >90% of healthy volunteers who received a dose of Orochol® (5×10^8 CFU). Orochol® was sold in 17 countries other than Australia, with over 500,000 doses distributed worldwide and had no significant adverse events reported.

104. Safety of the newly manufactured GM cholera vaccine Vaxchora® has been extensively tested in several human clinical trials (~3,563 individuals). Vaxchora® is found to be safe, well tolerated and elicited serum antibody responses in vaccine recipients (Levine et al., 2017; Mosley et al., 2017; McCarty et al., 2018). No significant adverse events were reported throughout the course of the studies. Further, safety data from 70,041 doses distributed between June 2016 and September 2018 in the US during the post-marketing phase of Vaxchora® is available (Discussed in Chapter 1, Section 6.2).

Section 5 The receiving environment

105. The receiving environment forms part of the context for assessing risks associated with dealings with GMOs (OGTR, 2013). It informs the consideration of potential exposure pathways, including the likelihood of the GMOs spreading or persisting outside the site of release.

5.1 Site of release

106. The primary environment receiving the GM cholera vaccine would be the gastrointestinal (GI) tract of the vaccine recipient.

107. The principal route by which the GM bacteria may enter the wider environment following vaccination is via shedding. Further, GM bacteria may also enter the environment via accidental spilling of unconsumed vaccine.

108. Human gut microbiota is excreted into sewage and wastewater, where it is removed through standard waste treatment processes, prior to the water being released back into the environment. The sewage treatment is also likely to be effective at removing the GM bacteria from sewage. However, due to variable levels of sewage treatment in wastewater plants (Toze et al., 2012), this could result in varying amount of bacteria in sewage and could result in release of some GM bacteria directly into rivers or marine environments.

5.2 Presence of related bacterial species in the receiving environment

109. Wild-type cholera toxin producing *V. cholerae* exist in the Australian environment. The first reported case of cholera in Australia was acquired from the Australian aquatic environment in 1977 (Desmarchelier et al., 1995). 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., 1995).

110. Since 1991, the majority of cholera cases have been acquired outside Australia and brought into Australia with exception of 1 case which was acquired in a laboratory and 3 cases in 2006 which were associated with the consumption of imported raw white bait (Forssman et al., 2007; Queensland Health, 2015; Australian Immunisation Handbook, 2018).

111. An average of 3 cases of cholera per year have been detected in people returning from cholera-affected countries (Forssman et al., 2007; Queensland Health, 2015; Australian Immunisation Handbook, 2018; Department of Health, 2020).

112. There are also rare sporadic occurrences of cholera in NSW and Queensland where the organism is found in some river systems.

113. 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 and Klose, 2002). Non-O1 *V. cholerae* are also present in Australian waters and may cause mild diarrhoeal disease but are not regarded as a significant public health hazard.

114. Cholera is a notifiable disease in all states and territories in Australia and cholera affected people are quarantined.

5.3 Presence of similar genes and encoded proteins in the environment

115. 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).

116. 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., 1999), particularly where the presence of mercury contamination provides positive selection pressure (Boyd and Barkay, 2012; Freedman et al., 2012; Møller et al., 2014).

117. The source of the *mer* operon used in the GM bacteria, *S. flexneri*, 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 (Queensland Health, 2018; Department of Health, 2020). Transmission of a multiple drug resistance plasmid, which also contains the *mer* operon, between *S. flexneri* and *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 6 Previous authorisations

6.1 Australian authorisations

118. Commercial supply of the GM bacteria i.e., *V. cholerae* CVD-103 HgR as a human vaccine (formerly known as Orochol®) was previously approved in Australia. The GMO described in this application is the same GM bacteria as previously approved GM cholera vaccine Orochol®.

119. Orochol® was approved by the Genetic Manipulations Advisory Committee (GMAC) on 24 November 1999.

120. Orochol® was registered as a prescription medicine by the TGA approval under the *Therapeutic Goods Act 1989* on 17 April 2000 after rigorous evaluation.

121. With the establishment of the gene technology legislation in 2001 and in accordance with section 190 of the Act, a 'deemed' licence for Orochol® was issued followed by a commercial licence approved under DIR-033 on 20 June 2003. The licence was surrendered on 14 September 2010 for commercial reasons.

122. Over 80, 000 doses of Orochol® were distributed in Australia between 2 September 2000 and 20 June 2003. However, no vaccine was sold under DIR-033. There were only two suspected adverse events reported in Australia in relation to Orochol®.

123. The GM cholera vaccine containing the same GM bacteria is now manufactured by Emergent Biosolutions under the trade name Vaxchora®. Phase III clinical trials for Vaxchora® (then called PXVX0200) were conducted in Australia to confirm the safety and efficacy of the newly manufactured vaccine. These trials were authorised under a licence issued on 10 April 2014 by the Regulator (DIR-126). The licence was surrendered on the licence holder's request on 10 September 2020.

6.2 International authorisations and experience

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

125. Orochol® was first registered in Switzerland in 1993 and was available until its production was stopped for commercial reasons in 2004. Worldwide over 500,000 doses of Orochol®were sold in total.

126. Vaxchora® is currently approved for use in the following regions:

Table 1 Overseas marketing approvals for Vaxchora®

Registered in:	Registration No.	Year of Registration	Age	Administration at:
United States	125597	2016	18-64 years	Healthcare facilities
European Union	EMEA/H/C/003876	2020	6-64 years	Home

127. Vaxchora® post market monitoring from the US has reported distribution of 70, 041 doses between June 2016 and September 2018. There were 20 incidents in 15 vaccine recipients reported as part of the pharmacovigilance reporting which included vaccine administration errors (such as consumption of food/drinks at inappropriate time points, administration of drug without buffer and administration of expired product) and reports of vaccine recipients feeling hot, rash, anxiety, fatigue, vomiting and blood in stool. None of these resulted in serious adverse events.

128. The applicant stated that there were no new safety issues identified in association with Vaxchora®.

Chapter 2 Risk assessment

Section 1 Introduction

129. 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 5). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

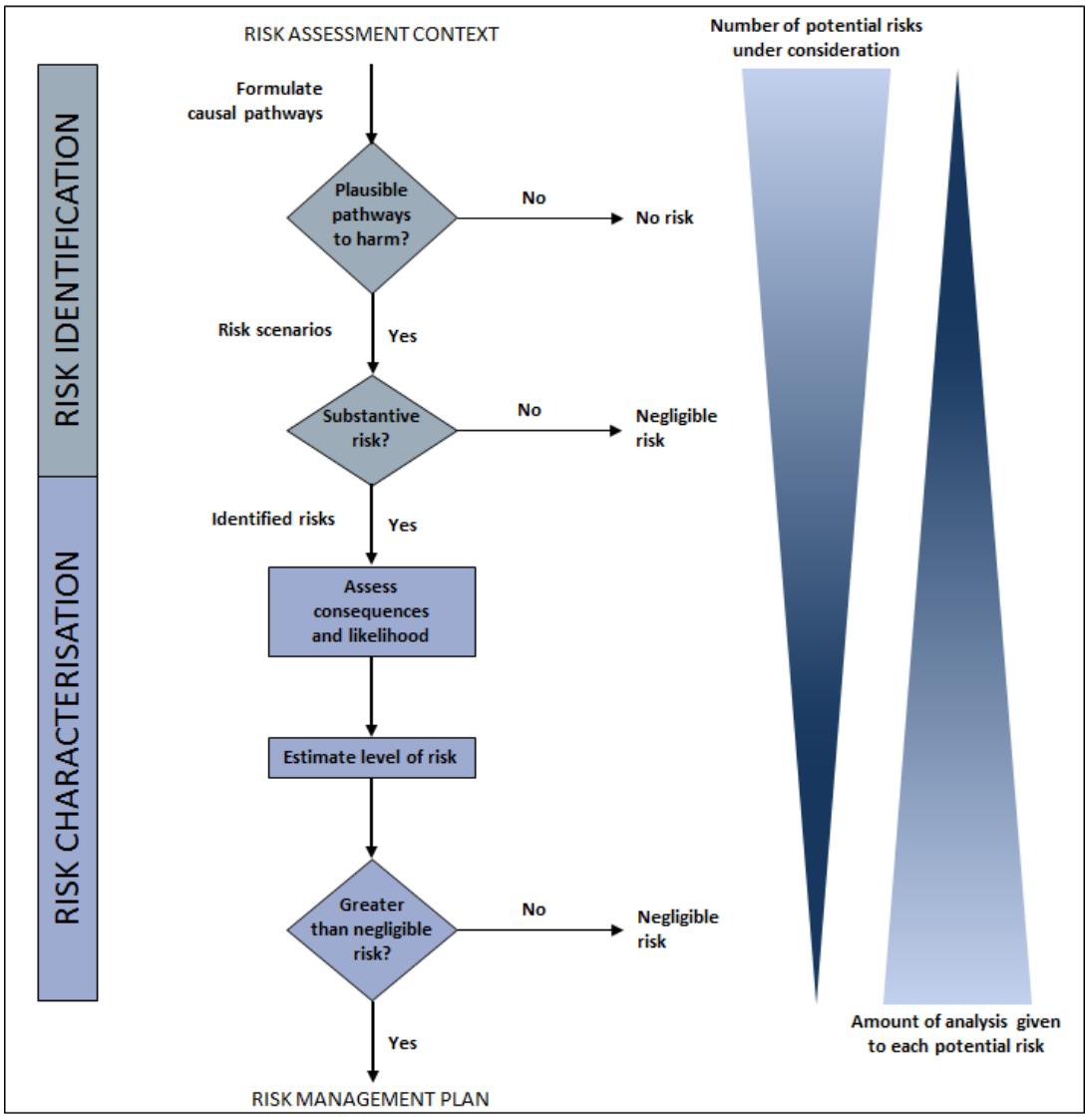

Figure 5. The risk assessment process

130. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

131. Risk identification first considers a wide range of circumstances in which the GMO, or the introduced genetic material, could come into contact with people or the environment. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

132. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 5), i.e. the risk is considered no greater than negligible.

133. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

134. Postulated risk scenarios are comprised of three components (Figure 6):

i. The source of potential harm (risk source)

ii. A plausible causal linkage to potential harm (causal pathway), and

iii. Potential harm to people or the environment.

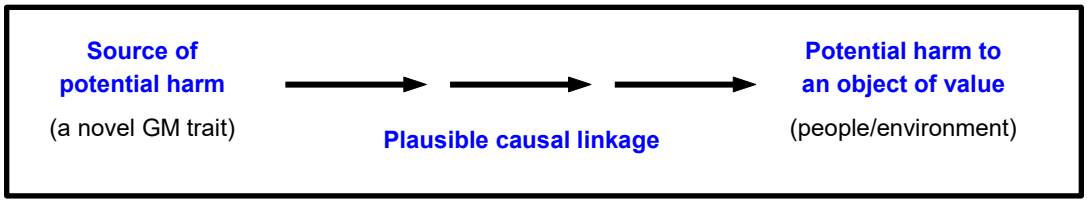

Figure 6. Components of a risk scenario

135. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMO and
- the characteristics of the parent organism(s).

2.1 Risk source

136. The parent organism of the GMO is the toxigenic bacterium *Vibrio cholerae* 569B. Details on the pathogenicity and transmissibility of *V. cholerae* is discussed in Chapter 1. Infection is generally the result of ingestion of food or water contaminated with the bacterium. Disease symptoms include profuse watery diarrhoea, vomiting and stomach cramps and in some cases cholera disease can be fatal.

137. Potential sources of harm can be intended novel GM traits associated with one or more of the introduced genetic elements or unintended effects/traits arising from the use of gene technology. Unintended effects can arise through horizontal gene transfer (HGT), the stable transfer of genetic material from one organism to another without reproduction. All genes within an organism, including those introduced by gene technology, can be transferred to another organism by HGT. A gene transferred through HGT could confer a novel trait to the recipient organism. The novel trait may result in negative, neutral or positive effects on the fitness of the recipient organism.

138. As discussed in Chapter 1, Section 4.1, the GM bacteria has been modified by the deletion of cholera toxin A subunit gene and insertion of mercury resistance operon into the haemolysin A gene. These introduced genes and their encoded proteins are considered further as a potential source of risk.

139. The current assessment focusses on risks posed to people or the environment, including long term persistence of the GMOs, which might arise from the import, transport, storage or disposal of Vaxchora®.

2.2 Causal pathway

140. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- the proposed dealings, which are import, transport or disposal of Vaxchora® and possession (including storage) in the course of any of these dealings,
- restrictions placed on the import, transport or disposal of Vaxchora® by other regulatory agencies, the States and Territories,
- characteristics of the parent organism,
- 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) on the properties of the organism,
- potential exposure of other organisms to the introduced gene(s) and gene product(s) from other sources in the environment,
- potential exposure of other organisms to the GMOs in the environment,
- the release environment,
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential),
- environmental stability of the organism (tolerance to temperature, UV irradiation and humidity),
- gene transfer by horizontal gene transfer,
- unauthorised activities, and
- practices before and after administration of Vaxchora®.

141. The TGA regulate quality, safety and efficacy of Vaxchora® under the *Therapeutic Goods Act 1989*, as mentioned in Chapter 1, Section 1.1. This includes:

- assessment of patient safety, vaccine quality and efficacy prior to inclusion on the ARTG,
- recommended practices for the transport, storage and disposal of the GM vaccine under the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8,*
- requirements for the scheduling, labelling and packaging under the *Poisons Standard.*

142. The current assessment focuses on risks posed to people or the environment, including long term persistence of the GMOs, which may arise from the import, transport, storage or disposal of Vaxchora®.

143. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

144. As discussed in Chapter 1, Section 3.3.3, TCP is the primary host range determinant for toxigenic *V. cholerae* (Kaper et al., 1995) as it mediates bacterial cell binding to host cells and therefore allows for colonisation of the host gut. TCP in the GM bacteria has not been modified. As mentioned in Chapter 1, Section 4.2, loss of cholera toxin and haemolysin and the expression of the *mer* operon are not expected to change the host range of the GM bacteria. Therefore, GM bacteria would not be expected to colonise or have an adverse effect on animals or any other organisms in

the environment, other than humans. Thus, there is no potential risk of the GM bacteria to animals other than humans and therefore, this risk scenario will not be considered further.

2.3 Potential harm

145. In addition, the following factors are taken into account when postulating relevant risk scenarios for this licence application:

- harm to the health of people including disease in humans or adverse immune response
- the potential for establishment of the GM *V. cholerae* CVD 103-HgR strain in the environment

2.4 Postulated risk scenarios

146. Three risk scenarios were postulated and screened to identify substantive risk. These scenarios are summarised in Table 2 and discussed in depth in this Section 2.4.1-2.4.3 (this chapter).

147. In the context of the activities proposed by the applicant and considering both the short and long term, none of the three risk scenarios gave rise to any substantive risks that could be greater than negligible.

Table 2 Summary of risk scenarios from dealings with GM cholera vaccine

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
1	GM bacteria	Exposure of other people to the GMO via contact with abraded skin or mucous membranes, inhalation/ ingestion during (a) Preparation and administration of the GMO (b) Import, transport or storage of the GMO (c) Disposal of the GMO (d) Shedding ⬇ Colonisation of GM bacteria in the small intestine ⬇ Infection	Ill health	No	• The GM bacteria has been modified to prevent production of the cholera toxin and haemolysin proteins and is unable to cause disease. • Exposure leading to infection requires entry of GMO by ingesting large amounts of infectious GM bacteria. • Without the bicarbonate buffer, the GM bacteria is killed by the acids found in the human stomach and is also very susceptible to hot and dry conditions, therefore would not persist on contaminated surfaces. • Transport, storage and disposal of the GM vaccine would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*. • The dose received through accidental exposure would be far smaller than that administered during vaccination.

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
					• After vaccination, GMO is expected to be shed in far lower numbers than the originally administered dose and for a short period of time (maximum 14 days post vaccination). • Sewage treatment is common practice in most Australia cities and would kill the bacteria.
2	GM bacteria	Exposure of other people to the GMO as mentioned in Risk Scenario 1 ⬇ Colonisation of GM bacteria in the small intestine ⬇ (a) Reversion of the GMO to the toxigenic phenotype (b) Transfer of genetic material to or from the GMO ⬇ Infection	Cholera, Ill health	No	• The genome of the GM bacteria is fully sequenced and characterised. • Genetic stability of the GM bacteria has been investigated in several different studies and the GM vaccine was found to be stable. • Reversion of the GM bacteria to the toxigenic phenotype would not increase the pathogenicity of the microorganism above the parent strain. • The *mer* operon is incorporated into the chromosome of the GM bacteria and is found to be very stable.
3	GM bacteria	Release into the environment via shedding, accidental spill and unconsumed residues as per Risk scenario 1 ⬇ (a) GMO persistence in the environment (b) Transfer of genetic material to or from the GMO (c) Reversion of the GMO to the toxigenic phenotype ⬇ Exposure to contaminated water and/or food	Ill health, cholera	No	• Up to 11% of vaccine recipients are expected to shed small amount of GM bacteria in their faeces for about a week. • Reversion of the GM bacteria to the toxigenic phenotype would not increase the pathogenicity of the microorganism above the parent strain. • Persistence of GM bacteria is lower compared to the wild-type strains present in the environment.

2.4.1 Risk scenario 1

Risk source	GM bacteria
Causal pathway	Exposure of other people to the GMO via contact with abraded skin or mucous membranes, inhalation/ ingestion during (a) Preparation and administration of the GMO (b) Import, transport or storage of the GMO (c) Disposal of the GMO (d) Shedding ⬇ Colonisation of GM bacteria in the small intestine ⬇ Infection
Potential harm	Ill health

Risk source

148. The source of potential harm for this postulated risk scenario is the GMO i.e., GM bacteria.

Causal Pathway

149. People (staff/person administering the vaccine or handling the vaccine, household contacts including at risk people and pregnant women) can be directly or indirectly exposed to the GM bacteria in a number of ways. This exposure could result in colonisation of GM bacteria in the small intestine and subsequently, infection that could lead to ill health.

Exposure during preparation and administration

150. The GM bacteria can be transmitted to other people during preparation and administration of the GM cholera vaccine in a number of ways including

- by ingesting the vaccine residues from the sachet or from the items used to prepare the vaccine or by direct contact with contaminated surfaces,
- by touching face or eyes during reconstitution,
- spill of dried or reconstituted vaccine,
- spit/vomit immediately after vaccine administration.

Any exposure via these pathways would only involve exposure to low levels of the GMO which is unlikely to result in any negative effects or ill-health.

151. The following procedures are proposed to be included in the vaccine leaflet to provide information on the preparation and administration of the GM vaccine for the carers/vaccine recipients/medical staff:

- Active ingredient sachet and disposable items are to be disposed of in a household waste container (at home)/pathological waste (at medical facilities) followed by immediately washing of hands and practising good toileting hygiene process. If non-disposable items are used during preparation of the vaccine at home, these items are to be washed using a dishwasher or with hot water and soap.
- People are advised to not touch their face or eyes while preparing the vaccine and to wash hands thoroughly with soap and hot water to prevent contamination.

- If a spill occurs during preparation, stirring or reconstitution or if there are any residues left (powder or liquid) on the mixing surface, vaccine recipients and medical staff are advised to clean up the spilled material or residue using disposable paper towels or cloth soaked in hot water and soap or an antibacterial disinfectant followed by discarding the paper towel/cloth in the household waste (at home)/pathological waste (at medical facilities). Similarly, a spill clean-up procedure would be initiated in case of spill of the vaccine by a vaccine recipient or a vomit following vaccine administration.

These procedures would help to mitigate any effects of the GMO to potentially exposed persons.

152. As *V. cholerae* is acid-labile (pH sensitive), the GM bacteria would not survive in the gastric acid present in the stomach in the absence of the bicarbonate buffer. Thus, if the lyophilised form of GM bacteria is ingested it would be killed in the stomach and therefore GM bacteria will be unable to colonise the intestinal tract and unable to cause ill health.

Exposure during import, transport and storage of the GMO

153. If the GM vaccine was unintentionally/accidentally spilled or lost during import, transport or storage, this could result in exposure to people in the area, due to contact of mucous membranes/skin with contaminated surfaces and ingestion/inhalation of the GM bacteria, and subsequent infection with the GMO.

154. The vaccine will be transported and stored according to the National Vaccine Storage Guidelines: Strive for 5 (Department of Health, 2019) and the Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP, 2020). The cold chain, which is intended to preserve the potency of the vaccine, requires cold packaging/refrigeration and this adds a level of containment during import, storage and transport.

155. In addition, the lyophilized vaccine has reduced capacity to survive in the environment compared to bacteria found in biological specimens. Further, without the bicarbonate buffer the GM bacteria would have poor survival in the acidic conditions of the human gastrointestinal tract.

156. The GM cholera vaccines will also follow the *Australian code of good wholesaling practice for medicines in schedules 2, 3, 4 & 8* (NCCTG 2011) as it will be classified as Schedule 4 medicines. These practices would also minimise the chances of damaged and leaking stock going unnoticed and increase the chances of Vaxchora® being handled by individuals who would know how to decontaminate a spill, thus minimising the probability of unintended dispersal of the GMOs.

157. If the recipient has consumed food or water 60 minutes prior to vaccination, then the GM vaccine in its original package (double-containment) will be provided to the recipient and vaccine will be transported to the recipient's home. As mentioned above, if a spill occurs at home, people will be instructed to decontaminate the area with an anti-bacterial disinfectant and/or hot water and soap as the GMOs are susceptible to common chemical decontaminants such as detergents and hypochlorite.

158. The applicant proposed that the Vaxchora® Safety Data Sheet will contain a 24/7 emergency contact number for identified spills/accidents involving this vaccine and the contingency plan will also be available on Biocelect's website to minimise and monitor the risk associated with the spills.

159. The import, transport and storage procedures proposed by the applicant meet the requirements of the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* and would mitigate exposure due to spills of the GMO during these dealings.

Exposure during disposal of the GMO

160. Individuals may be inadvertently exposed to GMOs while disposing of used, expired, or unused cartons of Vaxchora®. The two locations where this is most likely to occur are at:

- distribution warehouses where stocks of Vaxchora® are held, and

- locations where Vaxchora® is administered.

161. In addition, exposure could also occur during disposal of nappies containing the GMO in the faeces or materials contaminated with the GMO.

162. As mentioned in Chapter 1, Section 2.1, the applicant has proposed that all unused and expired GM vaccine will be disposed of according to institutional procedures for disposal of expired drug stocks such as high temperature incineration at medical facilities/warehouse. Given the GMOs would still be a dry powder in a sealed sachet during waste disposal, the waste handlers are highly unlikely to be exposed to GM bacteria in a manner that would result in ill-health.

163. Following vaccine administration in medical facilities, used disposable cups and stirring items and empty vaccine sachets that contained GM bacteria would be immediately placed into pathological waste followed by decontamination by methods approved by the Environmental Protection Agency or Health Department in each State or Territory. The clinical waste stream is considered appropriate for disposal of the GM bacteria (Tasmania, 2007; Western Australia, 2016; Australian Capital Territory, 2017; New South Wales, 2018; Queensland, 2019; South Australia, 2020).

164. When the vaccine is administered at home, empty active ingredient sachet, empty buffer sachet and used disposable cups and items will be disposed of in household waste which will be carried to landfill where the GM bacteria will die off within a few days. If a non-disposable cup and stirring item are used in the preparation, they will be washed with hot soapy water or dishwasher which would kill the GM bacteria. This will reduce the exposure of unintended people to the GMO.

165. Taken together, these proposed disposal and decontamination procedures would minimise and control risks associated with conducting these dealings with the GMOs.

Exposure of people to the GMO due to shedding

166. A potential exposure to the GM bacteria to people may occur via potential shedding post vaccination. GM vaccine recipients could potentially discharge unincorporated inoculum or shed GMO which could contaminate surfaces with the GMO and could lead to infection of other people.

167. Based on the Orochol® and Vaxchora® shedding data (Chapter 1, Section 4.3.2), some level of shedding is expected from 10-30% of vaccine recipients for a maximum of 14 days. This level of shedding was observed from the vaccine recipients and did not result in household transmission. Therefore, people being accidentally exposed to a low dose of GM bacteria are not expected to shed GM bacteria for more than 14 days, however some level of shedding is expected to occur. It is possible that people may ingest GM bacteria that entered the environment via human waste. However, it is extremely unlikely that persons could ingest enough of the GM bacteria by this route to cause disease. In addition, transmission to household contacts was not detected in the previous study (Chen et al., 2014).

168. Treatment of wastewater including sewage is required as per State and Territory regulations and would reduce/limit the chances of GM bacteria entering into environmental waters. However, due to variability between wastewater treatment plants in their potential effectiveness to reduce bacteria (Toze et al., 2012), this could result in the presence of small number of GM bacteria in the sewage and eventually their entry into the environment.

169. Septic tanks, are used in some local areas where commercial wastewater treatment is not available. This could result in exposure of people to the GM bacteria via leakage of incompletely treated effluent. However, each State and Territory has regulations which require septic tanks to be maintained which reduces the chance of release of untreated sewage from the septic tanks. In addition, any spilt GM bacteria which comes into contact with surrounding soil due to septic tank leakage is unlikely to persist in the environment (Discussed further in Risk Scenario 3).

170. Nappies from children vaccinated with Vaxchora® are to be disposed of in household waste and carers are instructed to wash their hands thoroughly after handling nappies. This would reduce the contamination of surfaces and exposure of people to the GM bacteria present in the stool.

171. Standard hygiene practices and wastewater treatment measures are sufficient to minimise harms resulting from exposure of household contacts to the low level of GM bacteria excreted from the vaccine recipient.

Potential harm

172. Any dose received through accidental exposure would be substantially less than that administered to vaccine recipients and would not be expected to result in infection as a large dose of vaccine is required to establish a transient infection and could generate an immune response in response to the GM bacteria.

173. The GM bacteria cannot replicate well outside a host, is acid-labile and is readily decontaminated. Therefore, exposure to a small amount of vaccine without the buffer is unlikely to result in infection. In addition, the GM bacteria is non-toxic due to deletion of parts of the cholera toxin and haemolysin genes, and is less pathogenic compared to wild-type *V. cholerae* strains. Therefore, exposure of people to the GM bacteria would not result in an increased disease burden in humans.

174. In the unlikely case that the small amount of GM bacteria survives the acid in the stomach and colonises the intestinal tract, the individual may become positive for antibodies against *V. cholerae*. However these low level antibodies would not represent a harm. Post-marketing monitoring and clinical studies have shown that the vaccine strain CVD 103-HgR is safe and does not cause serious side-effects and potential immune response is not dangerous.

175. If GM bacteria is accidently ingested or other mucous membranes are accidently exposed to a GMO (i.e., the eyes/mouth are touched while preparing the vaccine), this could result in a local bacterial infection. The infection could be treated by commonly available antibiotics.

176. Low levels of GM bacteria is expected to be excreted from a few vaccine recipients for a short period of time. The minimal amount and transient nature of any infection resulting from unintended exposure would be expected to result in very mild, or no symptoms.

Conclusion

177. The potential for an unintentional exposure of people to the GM cholera vaccine resulting in increased disease burden in humans is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

Risk source	GM bacteria
Causal pathway	Exposure of other people to the GMO as mentioned in Risk Scenario 1 ⬇ Colonisation of GM bacteria in the small intestine ⬇ (a) Reversion of the GMO to the toxigenic phenotype (b) Transfer of genetic material to or from the GMO ⬇ Infection
Potential harm	Cholera and/or Ill health

Risk source

178. The source of potential harm for this postulated risk scenario is the GMO i.e., GM bacteria.

Causal Pathway

179. The transmission of GM bacteria can occur by the pathways mentioned in Risk Scenario 1 which could potentially result in transient colonisation of GM bacteria in the small intestine of humans. This colonisation could potentially revert the GM bacteria to the toxigenic phenotype and/or result in transfer of genetic material to or from the GM bacteria in the gastrointestinal tract causing infection.

180. As mentioned in Chapter 1, section 3.3.5, there are three major mechanisms by which bacteria can exchange genetic information by horizontal gene transfer i.e., conjugation, transformation and transduction. *V. cholerae* has been shown to exchange genetic material by all three mechanisms. Therefore, the GM bacteria could potentially revert back to a toxigenic phenotype by acquiring the *ctxA* gene and/or the *hlyA* gene in the gastrointestinal tract of humans. In addition, the genetic material and antibiotic resistance genes could be transferred between the vaccine strain and other competent bacterial species present in the gastrointestinal tract.

Reversion of the GMO to the toxigenic phenotype

181. The GM bacteria could revert to the toxigenic phenotype by regaining the ability to produce functional cholera toxin and/or functional haemolysin A protein by re-acquiring functional genes from wild-type cholera toxin producing *V. cholerae*. For the gene transfer events to occur, the person exposed to the GM bacteria would also need to be infected with the toxigenic or non-toxigenic *V. cholerae* strain at the same time.

182. The vaccinated individual could ingest the wild-type bacteria at an estuarine or marine settings e.g., via consumption of shellfish or surfing/swimming. Wild-type toxigenic strains of *V. cholerae* have been isolated from fish, shellfish and oysters in Australian waterways. Therefore, it is possible, albeit unlikely, infection with the wild-type strain could occur.

183. The chances of acquisition of cholera toxin genes and *hlyA* genes through horizontal gene transfer is also reduced because both genes are contained in a pathogenicity island on the chromosomes of the bacteria and exchange of chromosomal elements is uncommon. Further, the parent strain *V. cholerae* 569B, from which the GM bacteria was derived, does not produce any plasmids or lysogenic bacteriophages. The *ctxA* and *hlyA* genes are very stable and are not easily transferred.

184. No *ctxA* gene transfer between the GM bacteria and wild-type strains of *V. cholerae* was detected in the multiple experiments conducted to evaluate the frequency of *ctxA* gene transfer

(Kaper et al. 1994). In addition, varying the donor/recipient ratios, using broth instead of plate cultures and carrying out the experiments in marine water samples for 11 days and in the intestines of suckling mice also did not produce any evidence of horizontal gene transfer. Similarly, mating experiments between a *V. cholerae* strain harbouring the IncC plasmid and the GM bacteria did not detect gene transfer to the GM bacteria. Therefore, no evidence of reversion of GM bacteria to the toxigenic form was observed.

185. However, one *ctxA* gene transfer event was observed at a very low frequency (1.3×10^{-8}) when GM bacteria was mated with *V. cholerae* strain JBK56 which contains a P fertility plasmid (Kaper et al. 1994). These P plasmids are very rare in toxigenic strains, and their presence has been associated with reduced colonisation.

186. As mentioned in Chapter 1, Section 4.3.1, the genome of Vaxchora® has been fully sequenced and characterised. Sequence analysis of Orochol® isolated from stool samples of 11 trial participant showed no mutations either at the *ctxA* deletion site or at the *hlyA* deletion*/mer* insertion site which indicates high genome stability (Favre et al. 1996). Further, stability and homogeneity of 16 individual Vaxchora® colonies was assessed and all 16 colonies were found to be positive for the *mer* operon indicating the presence of high stability of the chromosomally integrated *mer* operon.

187. *In vitro* and *in vivo* studies showed no evidence that the vaccine strain could reacquire *ctxA* genes from wild-type *V. cholerae* O1 strains (Kaper et al., 1994; Favre et al., 1996). However, if the wild-type strains were genetically modified to add genes that promote chromosomal gene transfer, then *ctxA* sequences could be acquired by the vaccine strain. This data suggests that under strict specific circumstances, it is possible for the vaccine strain to reacquire the *ctxA* gene.

188. As mentioned in Chapter 1, Section 3.3.5, bacteriophages can integrate their genome into bacterial genomes, replicate inside a bacterial cell and produce progeny bacteriophages that can carry mobile genetic material from one bacterium to another. In some strains of *V. cholerae*, part of the genome that encodes the cholera toxin (*ctxAB*) genes also includes sequences (prophages) which if activated can produce infectious bacteriophages. This bacteriophage is able to transfer the *ctxAB* gene from *ctxAB* positive donor strains to *ctxAB* negative recipient strain.

189. Bacteriophage-mediated gene transfer of *ctxA* gene is highly unlikely as

- the vaccine strain is a non-permissive host for the bacteriophage
- the vaccine does not produce bacteriophages that are able to infect permissible *V. cholerae* strains;
- classical *V. cholerae* strains do not display a stable lysogenic (self-replicating element) state, therefore any resulting toxigenic strain would very rapidly return to a non-toxigenic state;
- lysogenic conversion by CTXϕ phages has not yet been observed outside the laboratory.

190. Although the molecular properties of the GM bacteria are well characterised, there is some residual chance that there could be other unexpected changes to the characteristics of the GM bacteria because of the genetic modifications.

191. There is a theoretical possibility that transfer of an active *hlyA* gene from a wild type El Tor strain to the vaccine strain could occur, in which case the vaccine strain could recover its ability to cause red blood cells to break apart. However, the inserted *mer* operon that is integrated into the chromosome of CVD 103-HgR replacing the *hylA* gene has been shown to be quite stable. Since the loss of the *mer* operon has never been observed, it is highly unlikely that a recombination event with a native El Tor strain would lead to recovery of haemolysin A activity.

192. Recombination events occur naturally for *V. cholerae* strains and the *mer* operon could theoretically be deleted from the vaccine strain CVD 103-HgR genome. However, recovery of the wild-type *hlyA* gene could not occur by mere deletion of the *mer* since a 400 bp fragment of the *hlyA* gene was also deleted during construction of CVD 103-HgR. Active *hlyA* could only be restored by

transfer of a wild-type *hlyA* gene from a wild type El Tor strain, not solely from a deletion of the *mer* operon. Therefore, the likelihood of these events occurring at the same time is highly unlikely.

193. The risk of reversion to a toxigenic strain through integration of functional cholera toxin genes and functional haemolysis genes is low due to the low baseline frequency of gene transfer events, low frequency of P plasmid in *V. cholerae* and the need for a toxigenic strain and the GM bacteria to be present in the gastrointestinal tract of the human host at the same time.

Transfer of genetic material to or from the GMO

194. The only foreign gene introduced in the GM bacteria (i.e., *V. cholerae* CVD 103-HgR) is the mercury-resistance locus (*mer* operon) inserted in the chromosomal *hlyA* locus. As mentioned in Chapter 1, Section 4.1, the *mer* operon occurs naturally in *S. flexneri* and other bacterial species. The *mer* operon does not encode any toxin or proteins known to cause significant adverse events. The *mer* operon is naturally present in the gut flora of the human gastrointestinal tract and is only expressed in the presence of mercury.

195. The stability of the *mer* operon after excretion following immunisation was examined in clinical trial participants. No genetic rearrangements occurred during the passage study or transit of the bacteria through the digestive tract at the *hlyA::mer* loci suggesting that *mer* operon is highly stable. Therefore, transfer of the *mer* operon to other bacterial species is unlikely.

196. In the GM bacteria, 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 bacteria to other micro-organisms (Ketley et al., 1993; Favre et al., 1996).

197. If transfer of the modified toxin genes from the GM bacteria to other bacterial species were to occur, the resulting hybrid would not be more harmful because the GM bacteria does not contain disease causing cholera toxin or *hlyA* genes. In addition, the disruption of *ctxA* and *hlyA* genes does not change the expression of other genes or result in the production of any novel toxic proteins or other toxic substances suggesting that there is no toxicity associated with addition of these genes into other bacterial species.

198. A large number of clinical trials of the GM cholera vaccine (previously known as Orochol®) and Vaxchora® involving thousands of subjects have been conducted. These trials have demonstrated that this vaccine is safe for human use and no significant toxicity or adverse events were attributed to the proteins of the *mer* operon in the clinical trials (Chen et al., 2014; Chen et al., 2016; Sow et al., 2017; McCarty et al., 2018; McCarty et al., 2019; McCarty et al., 2020) .

199. The *eltAB* locus encoding *E. coli* heat-labile toxin (LT) is present in enterotoxigenic *E. coli* (ETEC) strains on large F-like conjugative replicons (Ent plasmids) (Viret et al., 2004). Thus, another possible scenario to consider is the conversion of the vaccine strain to a toxin producing phenotype via the acquisition of an *eltAB* locus. The chances of co-infection of ETEC and vaccine strain is highly unlikely as the infection with ETEC causes diarrhoea like symptoms and would require immediate treatment. In the unlikely event, that the exchange of *eltAB* locus occurred, the ability for the vaccine strain to cause disease in human is limited due to unstable expression of these plasmids.

200. 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 unable to cause diarrhoea or unable to break down red blood cells (respectively). Similarly, transfer of modified genes from the GM bacteria to the non-toxigenic *V. cholerae* is not likely to result in an adverse outcome.

201. There is also a potential for transfer of antibiotic resistance genes from bacterial species present in the gut of a vaccinated individual to the GM bacteria resulting in a drug resistant GM bacteria.

Potential harm

202. If the person is infected with wild-type *V. cholerae*, the transfer of a functional *ctxA* gene from a *V. cholerae* strain to the GM bacteria could occur. This would result in the production of a toxigenic *V. cholerae* that is unable to break down red blood cells and would therefore still cause less severe disease than other *V. cholerae* present in the environment.

203. In the unlikely event that transfer of both functional *ctxA* gene and *hlyA* gene occurred in the human gastrointestinal tract, this could result in a toxigenic strain which could cause disease similar to the wild-type strain. This is not expected to increase the disease burden and can be readily treated by freely available antibiotics in Australia.

204. Transfer of *mer* operon from the GM bacteria to wild-type *V. cholerae* could potentially result in a toxigenic *V. cholerae* with mercury resistance which could provide an advantage to the bacteria in the presence of mercury.

205. If the GM bacteria were to acquire one or more antibiotic drug resistance genes, it may make the GM bacteria resistant to certain antibiotics and result in a multi-drug resistant bacteria in the gastrointestinal tract and eventually in the environment. However, since the GM bacteria lacks the genes which cause cholera disease (cholera toxin and haemolysin), the GM bacteria would be harmless to people but could result in multiple drug resistant GM bacteria into the environment. However, as there are already multi-drug resistant *V. cholerae* strains present in the environment (see Section 3.3.4), this change is not expected to increase the overall burden of disease.

206. If environmental bacteria with existing resistance to antibiotics were to also acquire the *mer* operon from the GM bacteria, this could result in highly resistant bacteria in the gastrointestinal tract and in the environment. Although unlikely to occur, the transfer of the *mer* operon could only provide a survival advantage to the bacteria if mercury was present. The addition of highly-resistant bacteria in the environment such as this, is therefore not expected to increase the overall burden of disease because mercury resistance is already the most wide-spread of all antimicrobial resistance determinants and has been found in a wide variety of bacteria from environmental and human sources (Chapter 1, Section 5.3).

Conclusion

207. The risk of exposure of people to the GMO which has acquired disease associated genes from other *Vibrio* species or other competent bacterial species is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

2.4.3 Risk scenario 3

Risk source	GM bacteria
Causal pathway	Release of GMO into the environment via shedding, accidental spill and unconsumed residues ⬇ (a) Persistence of GMO in the environment (b) Transfer of genetic material to or from the GMO (c) Reversion of the GMO to the toxigenic phenotype ⬇ Exposure to contaminated water and/or food
Potential harm	Cholera and/or Ill heath

Risk Source

208. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

209. GM bacteria could be released into the environment via shedding, accidental spill and unconsumed vaccine residues where it could result in GMO persistence in the aquatic environment, transfer of genetic material to or from the GMO and reversion of the GMO to the toxigenic phenotype. This release of GM bacteria could further result in infection in people due to exposure of people to contaminated water and/or food.

Persistence of GM bacteria in the environment

210. As mentioned in Chapter 1, Section 4.3.2 and Risk Scenario 2, ~11% of vaccine recipients shed GM bacteria in their stool after vaccination with Vaxchora®. However, these GM bacteria were not detected in sewerage drains near the houses of vaccine recipients (Chen et al., 2014).

211. In Australia, wastewater treatment including sewage is required as per State and territory regulations and is carried out in local government areas (Western Australia, 2004; Queensland, 2020; South Austalia, 2020; South Australia, 2020; Victoria, 2020). This is expected to reduce the amount of GM bacteria entering into the environmental waters and, if any, only a small number of the GM bacteria might enter in the environment.

212. It is also possible that in areas where there is limited sewerage treatment (such as septic tanks) and vaccinated individuals excreting GM bacteria directly into the environment (i.e., during camping or holidaying in coastal areas), there is a possibility for small number of the GM bacteria to enter the environment. This has the potential to result in persistence of GM bacteria in the environment in the dormant state and/or in association with aquatic animals or structures.

213. Laboratory studies have demonstrated that GM bacteria are not expected to multiply in the environment after excretion from vaccinated individuals. As mentioned in Chapter 1, Section 4.3.3, GM bacteria survives best in sterilised estuarine water in the absence of competition with other micro-organisms (10^3-fold decrease after 33 days). Furthermore, GM bacteria (2.2 x 10^5 CFU and 10^6 CFU) were not recovered from non-sterile estuarine water after 14 days and from soil after 19 days. These studies demonstrate that the GM bacteria do not multiply in soil or estuarine water when competition from other bacteria occurs however, these studies did not test for the presence of GM bacteria that may have entered VBNC state.

214. The GM bacteria is similar to the parent organism in terms of survival in the environment and ability to enter VNBC state. Therefore, there is a chance for GM bacteria to enter VBNC state and to form biofilms which can then be dispersed in the aquatic environment.

215. GM bacteria (*V. cholerae* CVD 103-HgR) does not contain any disease associated genes. In addition, wild-type *V. cholerae* (both toxigenic and non-toxigenic) strains are already present in small numbers in tropical and temperate aquatic environment. Therefore, addition of a small number of non-toxigenic GM bacteria persisting in the environment would not increase risk compared to the wild-type strain already present.

216. Expression of the *mer* operon genes would only provide an advantage in 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 bacteria. 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 GM bacteria would allow them to outcompete other bacterial species present in a mercury polluted environment.

217. Aquatic species can provide a mode of transport to the GM bacteria (see Chapter 1, Section 3.3.2) and could spread the GM bacteria in the environment. This could result in their survival and persistence in the environment.

Transfer of genetic material to or from the GMO

218. Transfer of genetic material between the GM bacteria and other competent bacterial species and vice versa can occur in the environment similar to the human gastrointestinal tract as discussed in Risk Scenario 2.

Reversion of the GMO to the toxigenic phenotype

219. As discussed in Risk Scenario 2, reversion of the GMO to the toxigenic phenotype can occur in a similar way in the environment but is limited by the requirement for acquisition of disease associated genes from a compatible donor.

220. Exchange of genetic material in soil or water microbes can occur via phage induction. However, the vaccine strain cannot make phage which can infect other strains as the vaccine strain is a non-permissive host for bacteriophages (Favre et al., 1996).

221. *V. cholerae* has been found in the gastrointestinal tract of aquatic species (e.g. copepods). Therefore, it is possible for these aquatic species to take up GM bacteria which has been released into the environment. This could result in the presence of both wild-type and vaccine bacteria in the aquatic species where gene transfer can occur and result in a toxigenic strain.

222. As mentioned in Chapter 1, Section 3.3.2*,* chitin increases the ability of *V. cholerae* to transfer genetic material and high numbers of *V. cholera* are often found during phytoplankton blooms (Mouriño-Pérez et al., 2003; Udden et al., 2008; Sinha-Ray et al., 2019). Under these circumstances, it is possible that the GM bacteria could acquire genes and transform from a non-toxigenic strain to a toxigenic strain.

223. Sunlight is also a potent inducer of transduction (lateral gene transfer) and may represent a natural mechanism for gene transfer of the CTX phage in the aquatic environment and for the generation of new toxigenic strains (Faruque et al., 2000; Chowdhury et al., 2017). Experimentally, these conversions were observed for only El Tor biotype and not for classical biotype such as the vaccine strain. Further, phage encoded exotoxin genes are widespread in terrestrial and aquatic environments (Casas et al., 2006). Therefore, there is a remote possibility for phages to provide new toxin or other genes to the vaccine strain if they are able to infect the vaccine strain.

224. As mentioned in Chapter 1, Section 3.3.5 and risk Scenario 2, the GM bacteria are unable to produce bacteriophages which can infect other bacteria.

225. If present in the environment, GM bacteria do not multiply (see Section 3.3.2) but may enter a VBNC state which are resistant to infection by bacteriophages.

226. The classical *vibrio* such as the GM bacteria are less likely to undergo horizontal gene transfer because they do not readily adhere to chitin or form biofilms compared to El Tor *vibrios* (Viret et al., 2004). Therefore, the possibility of the GM bacteria to persist in the environment is low compared to the other strains.

Potential harm

227. The host range of *V. cholerae* is strictly limited to humans therefore any potential release of the GM bacteria into the environment is not expected to have any direct impact on other organisms.

228. The persistence of GMO in the environment would not be expected to increase the disease burden in humans as the vaccine strain does not cause disease due to deletion of the cholera toxin and haemolysin genes.

229. The reversion of GMO to the toxigenic phenotype is low but if it happens, the GMO will cause a disease similar to the parent organism which is already found in the Australian environment. The disease would be then treated with readily available antibiotics.

Conclusion

230. The potential of GM bacteria to be released into the environment is not identified as a risk that could be greater than negligible. Therefore, it does not warrant further assessment.

Section 3 Uncertainty

231. Uncertainty is an intrinsic part of risk analysis[6]. There can be uncertainty in identifying the risk source, the causal linkage to harm, the type and degree of harm, the likelihood of harm or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

232. There are several types of uncertainty in risk analysis (Clark and Brinkley, 2001; Hayes, 2004; Bammer and Smithson, 2008). These include:

- uncertainty about facts:
  - knowledge – data gaps, errors, small sample size, use of surrogate data
  - variability – inherent fluctuations or differences over time, space or group, associated with diversity and heterogeneity
- uncertainty about ideas:
  - description – expression of ideas with symbols, language or models can be subject to vagueness, ambiguity, context dependence, indeterminacy or under-specificity
  - perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

233. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important to estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

234. Uncertainty can also arise from a lack of experience with the GMO itself. However since the GM cholera vaccine has been widely used for several years in several countries including Australia and is currently commercially available in the US and the EU. The overall level of uncertainty is low and no significant areas of uncertainty were identified.

235. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

236. Post release review (Chapter 3, Section 4) will be used to address uncertainty regarding future changes to knowledge about the GMO. This is typically used for commercial releases of GMOs, which generally do not have limited duration.

Section 4 Risk evaluation

237. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate

[6] A more detailed discussion is contained in the Regulator's *Risk Analysis Framework* available from the OGTR website or via Free call 1800 181 030.

or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

238. Factors used to determine which risks need treatment may include:

- risk criteria,
- level of risk,
- uncertainty associated with risk characterisation, and
- interactions between substantive risks.

239. Three risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether people and animals can be inadvertently exposed to the GMO, the potential for the reversion of GMO to the toxigenic phenotype and the potential for transfer of genetic material between GMO and other bacterial species. The potential for GM bacteria to be released into the environment and its effects was also considered.

240. A risk is substantive only when the risk scenario may, because of gene technology, have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance in the risk assessment process.

241. In the context of the control measures proposed by the applicant and the operating guidelines of the pertinent regulatory agencies, and considering both the short and long term, none of these scenarios was identified as representing a substantive risk requiring further assessment. The principal reasons for this include:

- the genetic modifications make the GMO unable to cause disease in people;
- *V. cholerae* does not cause disease in animals other than humans;
- the consequences of accidental exposure to GM bacteria by non-vaccines are negligible;
- the likelihood of reversion of GM bacteria to a toxigenic strain is very low and persistence of small numbers of GM bacteria in the environment would pose no additional risks to those posed by toxigenic *V. cholerae* already present in Australian aquatic environment;
- the product of the introduced *mer* operon gene resulting in mercury resistance is not expected to be toxic to humans and are already widespread in the environment, therefore it poses no additional risks to the health and safety of humans or the environment.

Therefore, any risks to the health and safety of people, or the environment, from the proposed commercial supply of the GM cholera vaccine are considered to be negligible. The *Risk Analysis Framework* (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. No controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment[7].

[7] 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 has allowed up to 6 weeks for the receipt of submissions from prescribed experts, agencies and authorities and the public.

Chapter 3 Risk management plan

Section 1 Background

242. 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 addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

243. 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 can be managed in a way that protects the health and safety of people and the environment.

244. 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 must also be reported to the Regulator.

245. 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 and to manage risk to people or the environment. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

246. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed supply of the GM cholera vaccine, Vaxchora®. These risk scenarios were considered in the context of the proposed receiving environment and the Australia-wide release, and considering both the short and long term. The risk evaluation concluded that no specific risk treatment measures are required to treat these negligible risks.

Section 3 General risk management

247. All DIR licences issued by the Regulator contain a number of conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- testing methodology
- identification of the persons or classes of persons covered by the licence
- reporting structures
- access for the purpose of monitoring for compliance.

3.1 Applicant suitability

248. 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

- 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.

249. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any circumstances that would affect their suitability.

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

3.2 Testing methodology

251. If a licence were issued, Biocelect would be required to provide a method to the Regulator for the reliable detection of the GMO, and the presence of the introduced genetic materials in a recipient organism. This instrument would be required prior to conducting any dealings with the GMO.

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

252. If a licence were issued, any person, including the licence holder, could conduct any permitted dealing with the GMO.

3.4 Reporting requirements

253. If issued, the licence would oblige 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 dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the release.

254. The licence holder is also obliged to submit an Annual Report containing any information required by the licence.

255. There are also provisions that enable the Regulator to obtain information from the licence holder relating to the progress of the commercial release (see Section 4, below).

3.5 Monitoring for compliance

256. 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 the Regulator, inspectors or other person authorised by the Regulator, to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

257. 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 the health and safety of people or the environment could result.

Section 4 Post release review

258. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator takes account of the likelihood and impact of an adverse outcome over the foreseeable future, and does not disregard a risk on the basis that an adverse outcome might only occur in the longer term. However, as with any predictive process, accuracy is often greater in the shorter rather than longer term.

259. For the current application for a DIR licence, the Regulator is has incorporated a requirement in the licence for ongoing oversight to provide feedback on the findings of the RARMP and ensure the outcomes remain valid for future findings or changes in circumstances. If a licence was issued, this ongoing oversight would be achieved through PRR activities. The three components of PRR are:

- adverse effects reporting system (Section 4.1)
- requirement to monitor specific indicators of harm (Section 4.2)
- review of the RARMP (Section 4.3).

The outcomes of these PRR activities may result in no change to the licence or could result in the variation, cancellation or suspension of the licence.

4.1 Adverse effects reporting system

260. Any member of the public can report adverse experiences/effects resulting from an intentional release of a GMO to the OGTR through the Free-call number (1800 181 030), mail (MDP 54 – GPO Box 9848, Canberra ACT 2601) or via email to the OGTR inbox (ogtr@health.gov.au). Reports can be made at any time on any DIR licence. Credible information would form the basis of further investigation and may be used to inform a review of a RARMP (see Section 4.3 below) as well as the risk assessment of future applications involving similar GMOs.

4.2 Requirement to monitor specific indicators of harm

261. Collection of additional specific information on an intentional release provides a mechanism for 'closing the loop' in the risk analysis process and for verifying findings of the RARMP, by monitoring the specific indicators of harm that have been identified in the risk assessment.

262. The term 'specific indicators of harm' does not mean that it is expected that harm would necessarily occur if a licence was issued. Instead, it refers to measurement endpoints which are expected to change should the authorised dealings result in harm. Should a licence be issued, the licence holder would be required to monitor these specific indicators of harm as mandated by the licence.

263. The triggers for this component of PRR may include risk estimates greater than negligible or significant uncertainty in the risk assessment.

264. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks greater than negligible. Therefore, they were not considered substantive risks that warranted further detailed assessment. Uncertainty is considered to be low. No specific indicators of harm have been identified in this RARMP for application DIR 174. However, specific indicators of harm may also be identified during later stages, e.g., through either of the other components of PRR.

265. Conditions have been included in the licence to allow the Regulator to request further information from the licence holder about any matter to do with the progress of the release, including research to verify predictions of the risk assessment.

4.3 Review of the RARMP

266. The third component of PRR is the review of RARMPs after a commercial/general release licence is issued. Such a review would take into account any relevant new information, including any changes in the context of the release, to determine if the findings of the RARMP remained current. The timing of the review would be determined on a case-by-case basis and may be triggered by findings from either of the other components of PRR or be undertaken after the authorised dealings have been conducted for some time. If the review findings justified either an increase or decrease in the initial risk estimate(s), or identified new risks to people or to the environment that require management, this could lead to changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

267. The risk assessment concludes that the proposed commercial release of GM cholera vaccine, Vaxchora® poses negligible risks to the health and safety of people or the environment as a result of gene technology.

268. The risk management plan concludes that these negligible risks do not require specific risk treatment measures. However, general conditions have been imposed to ensure that there is ongoing oversight of the release.

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Appendix A: Summary of submissions on RARMP preparation from experts, agencies and authorities

The Regulator received several submissions from prescribed experts, agencies and authorities[8] on matters relevant to preparation of the RARMP. All issues raised in submissions relating to risks to the health and safety of people and the environment were considered. These issues, and where they are addressed in the consultation RARMP, are summarised below.

Submission	Summary of issues raised	Comment
1	Confirms that the council has no advice or comment to provide on the application for a licence for the commercial supply of genetically modified cholera vaccine, Vaxchora.	Noted.
2	Council has reviewed the information provided and advises that it has no comment to provide on this matter.	Noted.
3	The Shire has no comment or concerns regarding this application.	Noted.
4	Please be advised that Council do not have this specialist scientific advice available to us so cannot make comment. Thank you for pre-consultation on the matter we have noted the details and await the outcome, if and when the proposed vaccine will be available.	Noted.
5	Thank you for the advice. Council has no further comment to make.	Noted.
6	Council has no advice to contribute at this time in relation to the proposed cholera vaccine.	Noted.
7	Noted	Noted.
8	Council does not have any comment on this matter but has referred it to QLD Health for comment.	Noted.
9	Council does not have a specialist scientific expert to make an assessment so no comments will be provided.	Noted.
10	No objection to, Vaxchora, with the positive benefits it brings to Australian travellers in cholera affected areas. We would support its administration through medical practitioners and the continued observational study on Vaxchora as is occurring in the EU.	Noted.
11	The Department agrees that the risks from the commercial release of this vaccine are negligible due to a number of factors. The Department agrees with the overall conclusions of previous RARMPs and the scientific application for DIR 174 and these will be directly relevant to the preparation of	Noted.

[8] Prescribed expects, agencies and authorities include GTTAC, State and Territory Governments, relevant local governments, Australian government agencies and the Minister for the Environment.

Submission	Summary of issues raised	Comment
	this RARMP. While there is no identified direct risk to the environment, it is recommended that in preparing the RARMP, additional discussion is included (detailed below) to support any risk assessment or conclusions regarding entry and persistence in the environment and potential for gene transfer.	Noted.
	Entry, survival and persistence in the environment. *Uncertainty regarding shedding duration and entry into the environment via sewage*	The potential of the GM bacteria to be released into the environment due to shedding is discussed in Chapter 2, Section 2.4.3 (Risk Scenario 3).
	Gene transfer and reversion to a pathogenic strain *Gene transfer in aquatic organism and the impact of environmental factors such as chitin and sunlight on gene transfer potential should be discussed in the RARMP.*	The potential of gene transfer in aquatic species, gene transfer due to environmental factors and the reversion of the GM bacteria to a pathogenic strain is discussed in Chapter 2, Sections 2.4.2 and 2.4.3 (Risk Scenarios 2 and 3).
12	The committee agrees that the following should be included in the RARMP: potential for reversion of the GMO to the pathogenic phenotype, potential accidental exposure of humans and other organism to the GMO resulting in harm and potential for GMO to be harmful to the environment	Noted.
	The committee also suggested to consider risks that may be related to persistence in biofilms and the potential for development of multidrug resistance in the RARMP.	Risks associated with persistence of biofilms and the potential for development of a multidrug resistance GMO is discussed in Chapter 2 (Risk scenario 2 and 3).
13	The information already available for Vaxchora should be thoroughly evaluated by the appropriate regulatory agency for safety and usefulness on the Australian population.	Noted.
	Should the vaccine be approved for use in Australia the below recommendations should be considered: 1. That the vaccine is not administered to children under 6 years of age as the efficacy and safety of the vaccine has never been established in children under 6 years of age. 2. Vaxchora has not been evaluated for genotoxicity or fertility impairment and therefore may pose a risk to the development of an unborn child in pregnant women. This needs to be considered along with administration of the	Risks associates with direct use of the vaccine would be considered by the TGA. Inadvertent exposure as a result of a spill during transport or storage, or during waste disposal was assessed to be a negligible risk.

Submission	Summary of issues raised	Comment
	vaccine to women who are breastfeeding and women who may be planning to become pregnant. 3. The vaccine study concluded that there is no immunogenicity or efficacy data in individuals over 64 years of age. The administration of the vaccine to individuals over the age of 64 also needs to be considered based on this statement. 4. If not already considered or in place, a screening checklist for cholera vaccine should be adopted. Attached is a sample checklist from the United States Defence Health Agency. It's a list of questions for providers to determine patients' eligibility to receive the cholera vaccine. 5. If the manufacture, bulk storage or disposal of the vaccine is to be conducted within our LGA, Council would like to be informed / consulted.	
14	This vaccine is the same as an earlier version that was approved before AND the cited approval for use in other geographical regions. The vaccine strain is unable to produce active cholera toxin or active haemolysin. I see no reason for any concerns about this application.	Noted.
15	No specific concerns with Biocelect proceeding with their licence application, including import, storage and disposal of the Vaxchora® vaccine in Australia.	Noted.
16	Broadly supportive and has no objections to application DIR 174.	Noted.
17	At this stage of the application process, no specific advice on risks to the health and safety of people and the environment to be considered in the development of the consultation RARMP.	Noted.

Appendix B: Summary of submissions from prescribed experts, agencies and authorities on the consultation RARMP

The Regulator received a number of submissions from prescribed experts, agencies and authorities 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.

Submission	Summary of issues raised	Comment
1	"The Shire does not have a position on the vaccine."	Noted.
2	"The Town has no current policy on GM product trials. However, the Town would like this to be undertaken in a way that is safe to both the public and the environment."	Noted.
3	"Council has no comment in relation to the commercial supply of GM cholera vaccine, Vaxchora. This vaccine will improve protection of Australian residents that intend travelling overseas to countries that have the cholera disease – it is designed to protect the health and wellbeing of people."	Noted.
4	"As the council does not have a specialist scientific expert to make an assessment, no comment will be provided."	Noted.
5	"The Regulator should consider including further information in the RARMP about risks associated with multi-drug resistance."	Additional consideration and discussion has been included in the RARMP about risk associated with development of multi-drug resistance bacteria (Chapter 2, Section 2.4.2 (Risk scenario 2)).
6	"Overall, Biocelect Pty Ltd's application has negligible risks to the health and safety of people and the environment. Specifically, the department is satisfied that the measures taken to manage the short- and long-term risks from the proposal are adequate."	Noted.
7	"The Department agrees with the overall conclusions of the RARMP that the direct risks to the environment or humans are likely to be negligible from shedding and release of the GM bacteria into the environment, due to the small-scale and minimal exposure of human hosts. However, as outlined in our previous advice, cholera bacteria are persistent in aquatic environments, and release into the environment as a causal pathway to potential harm should be assessed fully as has been done in previous RARMPs to support the conclusions of this RARMP."	Noted.

Submission	Summary of issues raised	Comment
	"Shedding *It should be made clear in the RARMP that it is unknown what percentage and for how long vaccinated individuals will shed GM bacteria, and therefore whether it will be present in household sewage."*	The RARMP has been modified to clarify the percentage and duration of shedding and the potential for their presence on the sewage (Chapter 1, Section 4.3.2 and in Chapter 2, Section 2.4.1)
	"Removal from sewage *It should be made clear in the RARMP that there is uncertainty regarding removal of bacteria from treated sewage and entry into the aquatic environment from treated sewage should be considered as a possible route of entry into the environment."*	Additional text has been added to the RARMP discussing the uncertainty regarding removal of bacteria from the sewage treatment and the potential of GM bacteria entering the aquatic environment (Chapter 1, Section 5.1 and in Chapter 2, Section 2.4 (Risk Scenarios 1 and 3)).
8	"This vaccine is release of a previously approved vaccine. There is an extensive history of the registration and use of the vaccine in a number of countries. On the basis of the above, I see no concerns about the proposed use of this vaccine in Australians travelling overseas."	Noted.
	"Both FDA https://www.fda.gov/vaccines-blood-biologics/vaccines/vaxchora (2018) and EMA https://www.ema.europa.eu/en/medicines/human/EPAR/vaxchora (2020) only approved the use in 18-64 (FDA) and 6+ (EMA) but product information states applicable to 2+. The Application does not clarify what age groups are the target audience of the supplier."	The vaccine is proposed to be administered to adults and children aged 2 years and older and has been discussed in Chapter 1, Section 2.
	"The information contained in DIR 174 Risk Assessment and Risk Management Plan (consultation version) supports the endorsement of application if adherence occurs to the following clause/s: • Product is to only be prescribed to 18-64 y.o. as there is insufficient information on the effect and efficacy of individuals of other ages in the application (none is provided). • Product is not to be prescribed to immunocompromised individuals. Product is to only be administered in medical facilities, not for administration in the home to ensure disposal of all product (including "left-over" mixed product) and efficacy."	The dealings regulated under the *Gene Technology Act 2000* include the import, transport, storage and disposal of the vaccine. The RARMP assessed risks to people as a consequence of conducting these activities and risks from persistence of the GM vaccine in the environment. The use of the vaccine and risk associated with direct use of vaccine will be assessed as part of the TGA assessment and requirements.
9	"Broadly supportive of application" and "no particular issues with the RARMP and believe the management plan proposed is consistent with risk."	Noted.
10	"Overall the members supported the Gene Technology Regulator's conclusion that the proposed supply of the	Noted.

Submission	Summary of issues raised	Comment
	GM cholera vaccine poses negligible risks to human health and safety and the environment."	
	"Vaxchora® appears low risk, given the similarities with Orochol®." "Suggest considering contingencies or restrictions to recipients, but also if recipients have household contacts that may be either immunocompromised, very young, or very old. Will require definition of target population, and caution for not only patient cohorts outside this range, but also strategy to mitigate risk to vulnerable household contacts."	The risks to human health and environment in the context of import, transport, storage and disposal are considered in the RARMP. In addition, risk to other people including immunocompromised people are considered in Risk scenario 1. Risks associated with direct use of the vaccine would be considered by the TGA in their assessment. The vaccine product information and consumer medicines information would contain information on the handling of the vaccine and ways to mitigate risks to vulnerable household contacts.
	Further consideration of: • "The person taking the vaccine may be already infected with V cholerae and could potentially revert back to a toxigenic phenotype by acquiring ctxA gene and-or hlyA gene in the gastrointestinal tract of humans. This does assume that the resistant bacteria would not be as virulent as V cholerae, however it does not provide a solution if it were to occur." • "Genetic material and antibiotic resistance genes could be transferred between the vaccine and other competent bacterial species present in the gastrointestinal tract. However, there is no risk discussion if bacterium other than Vibrio species develop multi-drug resistance strains due to the mercury resistance (mer operon) component of the Vaxchora® translating to other Gram-negative and Gram-positive bacteria."	The potential for development of a toxigenic *V. cholerae* has been discussed in Chapter 2, Section 2.4.2. The RARMP also discusses the treatment of these toxigenic *V. cholerae* with available antibiotics in Australia. The risk associated with transfer of mer operon to other bacterial species resulting in multi-drug resistant bacteria is now discussed in Risk scenario 2.
	What is being recommended in Australia in relation to administration of the vaccine?	Vaxchora® would be made available as a Schedule 4 prescription medicine and is proposed to be self-administered either at medical facilities or at home

Submission	Summary of issues raised	Comment
		in adults and children aged 2 years and older who would be visiting cholera-affected countries (Chapter 1, Section 2). The administration/use of the vaccine would be considered by TGA in their assessment.
	"How will information relating to adverse effects be relayed to the TGA? Will members of the public reporting adverse events via this pathway also be encouraged (or required) to report this information to the TGA?"	Adverse event reporting would also be considered by TGA under their assessment of the vaccine.

Appendix C: Summary of submissions from the public on the consultation RARMP

The Regulator received 2 submission from the public on the consultation RARMP. The issue raised in the submission is summarised in the table below. All issues 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.

Submission	Summary of issues raised	Comment
1	"I do not agree with genetic modification. Genetically modified is criminal and vaccines are even worse."	Noted.
2	"Madness!"	Noted.