Australian Government
Department of Health
Office of the Gene Technology Regulator

Risk Assessment and Risk Management Plan for

DIR 125

Commercial release of genetically modified vaccine to protect chickens against pathogenic *Escherichia coli*

Applicant: Zoetis Australia Research & Manufacturing Pty Ltd (Zoetis)

December 2014

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Summary of the Risk Assessment and Risk Management Plan

for

Licence Application No. DIR 125

Decision

The Gene Technology Regulator (the Regulator) has decided to issue a licence for this application for dealings with a genetically modified (GM) *E. coli* chicken vaccine. Zoetis Australia Research & Manufacturing Pty Ltd (Zoetis) has been approved under the *Gene Technology Act 2000* (the Act) to commercially release the GM chicken vaccine for the purposes of import, transport, storage and disposal within Australia. Subject to approval by other relevant authorities as set out below, Zoetis is permitted to import the GM chicken vaccine into Australia, and distribute it to commercial poultry farms.

Every veterinary vaccine for sale in Australia is required to be assessed for quality, safety and efficacy. The Australian Pesticide and Veterinary Medicines Authority (APVMA) administers the *Agricultural and Veterinary Chemicals Code Act 1994* to regulate agriculture and veterinary chemical products, including vaccines. Therefore, in addition to approval by the Regulator, Zoetis will require approval from APVMA for use of the GM vaccine.

Furthermore, import of the GM chicken vaccine is also subject to regulation by the Department of Agriculture which administers Australian biosecurity conditions for the importation of biological products under the *Quarantine Act, 1908*. These products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GM vaccines). Therefore, in addition to approval by the Regulator, Zoetis will require approval from the Department of Agriculture for import of the GM vaccine.

The Regulator has released a science based Risk Assessment and Risk Management Plan (RARMP) in accordance with the requirements of the Act and corresponding state and territory legislation, that was finalised following consultation with a wide range of experts, agencies and authorities, and the public. The RARMP concludes that this commercial release poses negligible risks to human health and safety and the environment. General licence conditions have been imposed for the release to ensure that there is ongoing oversight of the licence.

The application

Application number	DIR 125
Applicant	Zoetis Australia Research & Manufacturing Pty Ltd (Zoetis)
Project title	Commercial release of genetically modified vaccine to protect chickens against pathogenic *Escherichia coli*
Parent organism	*Escherichia coli* serotype O78, strain EC34195
Introduced or modified genes and resulting modified traits	Partial deletion of *aroA* gene (impaired biosynthesis of essential aromatic amino acids resulting in reduced spread and persistence of the GMO - attenuation)
Proposed locations	Commercial poultry farms in Australia
Proposed release date	Ongoing from date of approval
Proposed activities	Import, storage, transport and disposal of the GM chicken vaccine.

Risk assessment

The risk assessment concludes that there are negligible risks to the health and safety of people, or the environment, from the proposed commercial release, either in the short or long term. No controls are required to manage these negligible risks.

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMOs 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 current scientific/technical knowledge, information in the application and relevant previous approvals. Both the short and long term impact are considered.

Credible pathways to potential harm that were considered included whether changes in gene expression due to gene deletions could: result in products that are toxic or allergenic to people or other organisms; alter characteristics that may impact on the disease burden due to the GM *E. coli*; or produce unintended changes in bacterial characteristics. The chance for unintended exposure to the vaccine and the GM bacteria it contains, and for gene flow was also considered.

The principal reasons for the conclusion of negligible risks are that the genetic modification is unlikely to cause harm to people and the environment; the extensive previous experience with the GM chicken vaccine overseas, and bacteria similar to the GMO are common in the environment.

Risk management plan

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions.

As the level of risk is assessed as negligible, specific risk treatment is not required. However, the Regulator has imposed licence conditions under post-release review (PRR) to ensure that there is ongoing oversight of the release 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 new information about risks or unintended effects associated with the authorised dealings.

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Abbreviations

aorA	5-enolpyruvylshikimate 3-phosphate synthase
APEC	Avian pathogenic *E. coli*
APVMA	Australian Pesticides and Veterinary Medicines Authority
BIP	Biological Imports Program
BLASTn	Basic Local Alignment Search Tool nucleotide search
bp	Base pair of DNA
CCI	Confidential Commercial Information under section 185 of the *Gene Technology Act 2000*
CFU	Colony forming unit
DIR	Dealings involving Intentional Release
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
EU	European Union
ExPAC	Extraintestinal-pathogenic *E. coli*
FSANZ	Food Standards Australia New Zealand
GM	Genetically modified
GMO	Genetically modified organism
GTTAC	Gene Technology Technical Advisory Committee
HGT	Horizontal gene transfer
LGA	Local government area
NSW	New South Wales
OGTR	Office of the Gene Technology Regulator
RARMP	Risk Assessment and Risk Management Plan
the Act	The *Gene Technology Act 2000*
the Regulations	Gene Technology Regulations 2001
the Regulator	Gene Technology Regulator
USA	United States of America
USDA	US Department of Agriculture

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.
2. The Act in conjunction with the Gene Technology Regulations 2001 (the Regulations), an inter-governmental agreement and corresponding legislation that is being enacted in each State and Territory, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.
3. This chapter describes the parameters within which potential risks to the health and safety of people or the environment posed by the proposed release are assessed. The risk assessment context is established within the regulatory framework and considers application-specific parameters (Figure 1).

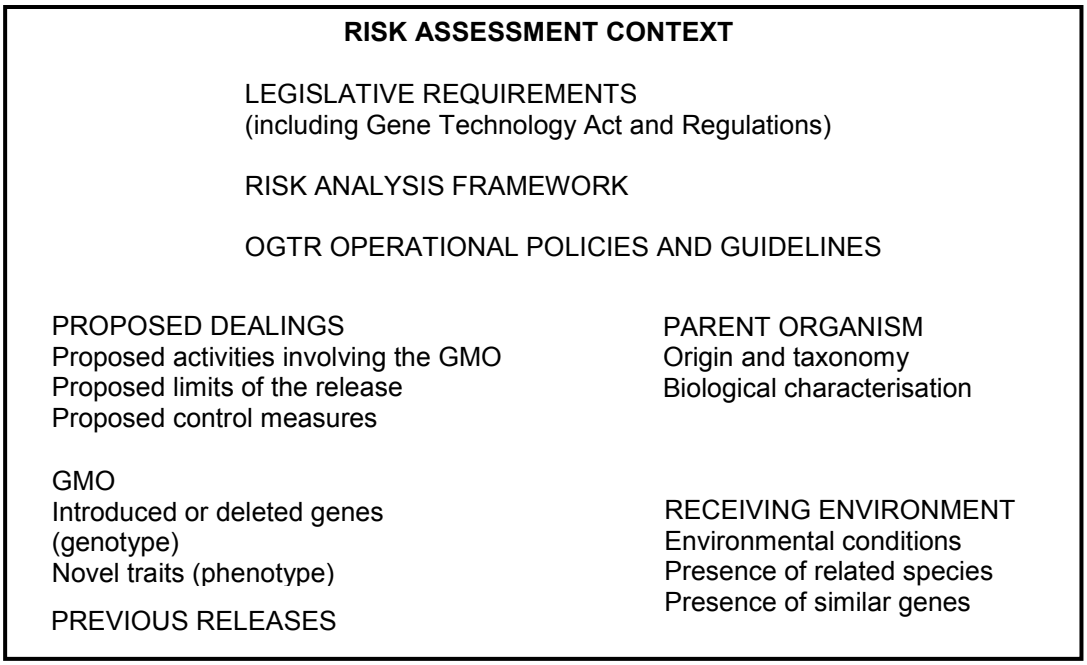

Figure 1. Summary of parameters used to establish the risk assessment context

Section 2 Regulatory framework

4. Sections 50, 50A and 51 of the Act outline the matters which the Gene Technology Regulator (the Regulator) must take into account, and consultation that is required when preparing the Risk Assessment and Risk Management Plans (RARMPs) that form the basis of decisions on licence applications. In addition, the Regulations outline matters the Regulator must consider when preparing a RARMP.
5. Since this application is not for experimental purposes, it cannot be considered as a limited and controlled release application under section 50A of the Act. This means that, under section 50(3) of the Act, the Regulator was required to consult with prescribed experts, agencies and authorities to seek advice 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, local councils and the

Minister for the Environment. A summary of issues contained in submissions received is given in Appendix A.

6. Section 52 of the Act required the Regulator, in a second round of consultation, to seek comment on the RARMP from the experts, agencies and authorities outlined above, as well as the public. Advice from the prescribed experts, agencies and authorities for the second round of consultation, and how it was taken into account, is summarised in Appendix B. Three public submissions were received and their considerations are summarised in Appendix C.
7. The Risk Analysis Framework explains the Regulator's approach to the preparation of RARMPs in accordance with the legislative requirements (OGTR 2013). Additionally, there are a number of operational policies and guidelines developed by the Office of the Gene Technology Regulator (OGTR) that are relevant to DIR licences. These documents are available from the OGTR website.
8. Any 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), Australian Pesticides and Veterinary Medicines Authority (APVMA), Therapeutic Goods Administration, National Industrial Chemicals Notification and Assessment Scheme and Department of Agriculture. These dealings may also be subject to the operation of State legislation declaring areas to be GM, GM free, or both, for marketing purposes.

Section 3 Regulation of the GMO and Proposed Dealings

9. Zoetis Australia Research & Manufacturing Pty Ltd (Zoetis) proposes to use Poulvac *E. coli*®, a live attenuated genetically modified (GM) *E. coli* bacteria, as a vaccine in commercially reared chickens. The GM chicken vaccine has been developed to protect chickens from *E. coli* O78 infection and is manufactured in the United Kingdom (UK).
10. Gene technology legislation operates in conjunction with other regulatory schemes in Australia.
11. The Australian Pesticide and Veterinary Medicines Authority (APVMA) administers the Agricultural and Veterinary Chemicals Code Act 1994 to regulate agriculture and veterinary chemical products, including vaccines.
12. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade. As part of the assessment of the GM vaccine, the APVMA will consider the risk posed by the presence of residual vaccine in meat and eggs of chickens.
13. Zoetis would require an approval from the APVMA to supply the GM vaccine. If approval is granted, the vaccine is likely to be classified as a prescription animal remedy and would require supervision by a registered veterinarian to be used in commercial poultry farms.
14. The Department of Agriculture administers Australian biosecurity conditions for the importation of biological products under the *Quarantine Act 1908*. These products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GM vaccines).
15. Although the use of the GM *E. coli* as a poultry vaccine would be regulated by the APVMA under *Agricultural and Veterinary Chemicals Code Act 1994*, its import, transport and disposal are subject to regulation under the Gene Technology Act. Import of

the GM vaccine is subject to co-regulation by the Department of Agriculture and the Regulator.

16. Therefore, the proposed dealings assessed by the Regulator are:
    - import
    - transport
    - disposal of the GM vaccine, and
    - possession (including storage) and supply of the GMO for any of the purposes above.
17. The GM vaccine would be transported as lyophilized powder in sealed glass or plastic vials by a commercial transport company, kept at 4°C and distributed to veterinary wholesalers, veterinarians and others involved in poultry production.
18. It is recommended by the applicant that persons administering the vaccine should wear the same personal protective equipment as used for preparation and administration of any other vaccine recommended for coarse spray to poultry. Lyophilised GM vaccine would be rehydrated in sterile water at 10,000 doses per litre and administered indoors. Disposal of unused or waste material would be done by boiling, incineration or immersion in an appropriate disinfectant. Disposal of litter would be according to standard commercial farm practice for disposal of used chicken litter (see chapter 1 section 6 and chapter 2 risk scenario 1 for more information).
19. The GM *E. coli* is attenuated by partial deletion of the gene *aroA*, which is involved in biosynthesis of essential aromatic amino acids. This restricts the ability of the GM vaccine to spread and persist. Therefore, the potential of the GM *E. coli* to cause disease in chickens is greatly reduced. The GM *E. coli* is proposed to be used as a poultry vaccine because it can help provide immunity to avian pathogenic *E. coli* (APEC) infection.
20. As the proposed release is not for experimental purposes, it is deemed a general/commercial release under the Act.

Section 4 The parent organism

21. The parent organism of the GM *E. coli* vaccine is *E coli* EC34195 (serotype O78) which can infect poultry, including chickens, and lead to respiratory distress, reduced appetite and poor growth. The parent organism was isolated in the UK, however the strain has also been found in chicken flocks in Australia (Murray 1987).

4.1 Biology of *E. coli*

22. *E. coli* are facultative anaerobic, gram negative, non-sporulating rod shaped bacteria. They typically inhabit the intestines and faeces of mammals, birds, amphibians and reptiles (Berg 1996; Gordon & Cowling 2003). Facultative anaerobes can survive both in aerobic as well as in anaerobic conditions. *E. coli* can be either non-motile or motile, with a peritrichous flagellum, and grow best at 37°C. Most strains are non-pathogenic and are commensal (Gordon & Cowling 2003) but some can cause diseases such as gastroenteritis, urinary tract infections, or sepsis (Dho-Moulin & Fairbrother 1999; Lister & Barrow 2008; Prescott et al. 2002).
23. Among birds and mammals, the probability of detecting *E. coli* increases with the size of the host (Gordon & Cowling 2003). This is likely due to the relationship between longer gut transition time and larger body size (O'Brien & Gordon 2011). In addition to host effects, gut morphology, gut dynamics and gut microbiota, background levels of *E. coli* are also important: *E. coli* is more likely to be recovered from birds, reptiles and frogs

living in association with humans compared to the same species living in isolation (Gordon & Cowling 2003).

24. The relationship between the host and *E. coli* is usually commensal. One of the two organisms benefits from the interaction between them whereas the other is neither harmed nor helped. *E. coli* strains derive a steady supply of nutrients as well as protection and dissemination from the host. This interaction, however, provides some benefits for the host as *E. coli* microbiota prevents colonizing and growth of pathogens by producing bacteriocins and other mechanisms (Hudault et al. 2001; Rastegarlari et al. 1990; Schamberger et al. 2004; Vollaard & Clasener 1994).

25. Commensal *E. coli* strains that reside in the digestive tract are located in the large intestine, especially in the caecum and the colon. The mucus layer covering the epithelial cells throughout the tract provides the main habitat for *E. coli*. They are shed into the intestinal lumen with degraded mucus components and are excreted in the faeces (Poulsen et al. 1994). *E. coli* can survive and transit in water and sediments. It is estimated that half of the *E. coli* population resides in these secondary habitats (Savageau 1983). The oral – faecal route is the main mode of transmission and distribution of *E. coli* and its presence in water is often used as an indicator of faecal pollution (Russell & Jarvis 2001; Savageau 1983).

26. *E. coli* has adapted to its ecological niche and competes with other bacteria in this niche for nutrients (Licht et al. 1999; Poulsen et al. 1994; Rang et al. 1999). This is demonstrated by a generation time of 30 minutes *in vitro* (no competition), 40 – 80 min in the intestines of streptomycin-treated mice (minimal competition) and 120 minutes after mice are 'conventionalized' by removing streptomycin and feeding of mouse caecal content (maximum competition) (Rang et al. 1999).

4.2 Genetics of *E. coli*

27. Very diverse bacteria are grouped into the species *E.coli.* Four main techniques have been used to identify and characterise *E. coli*. Serotyping was developed in the 1940s and is based on a surface antigen O, K and H. A very high number of serotypes have been described and this typing is still used today due to its robustness, simplicity and long history of use (Kauffman 1947; Orskov & Orskov 1992). Multilocus enzyme electrophoresis (MLEE, introduced in the 1980s), multilocus sequence typing (MLST, introduced in the late 1990s) and Phylogrouping triplex PCR (introduced in 2000) are the other techniques to study the genetic entities of *E. coli* (Clermont et al. 2000; Enright & Spratt 1999; Selander et al. 1986). The latter is used to assign *E. coli* to four main phylogenetic groups called A, B1, B2 and C and is widely used due to its simplicity and rapidity (Clermont et al. 2000).

28. The genome size varies widely across *E. coli* with the average genome containing around 5000 genes. Only 1700 genes are conserved among all strains (these are commonly referred to as 'strict core') and 3000 genes are conserved in at least 95% of the strains (commonly referred to as 'soft core') (Kaas et al. 2012). Hence each strain contains genes from the core genome and genes from an extended pool of genes of approximately 8000 genes. This provides a high level of plasticity in the genome and also reflects the adaptive nature of the organism (Tenaillon et al. 2010).

29. In addition to a large gene pool, *E. coli* is a conjugative bacterium that is capable of exchanging genetic elements with other compatible bacteria present in the surrounding environment. Genetic elements are thought to move horizontally (to compatible bacteria) and vertically (to offspring) as they can help bacteria adapt to changing environments (Kaper et al. 1995) and it contributes to the evolution of bacterial variants, resulting in the development of novel strains and pathotypes.

30. There are three main genetic mechanisms that enable the transfer of genetic elements in *E. coli*: transduction, transformation and conjugation. *Transduction* is the movement of genetic material with the help of bacteriophages. Erroneously packed host DNA can be transferred to another bacteria upon its infection with the phage. In theory, any region of the bacterial genome can be transferred in that way, including plasmids, but the DNA will not be retained by the host unless the phage integrate into the bacterial genome (prophage). The regions co-integrated with prophage DNA are commonly the flanking regions of the prophage insert site (Berg et al. 1983). *Conjugation* describes the direct transfer of DNA from one bacteria to another (Sorensen et al. 2005). This involves the formation of a plasmid encoded pilus, which then can be used by other plasmids or chromosomal regions to transfer genetic information across the cells. *Transformation* in *E. coli* involves the induction of competence, DNA binding followed by fragmentation of the DNA, uptake and stable maintenance of the DNA by either integration in the genome (recombination) or recircularization of plasmid DNA (Harrison & Brockhurst 2012; Mellata et al. 2010; Sorensen et al. 2005).

31. Another way by which *E. coli* bacteria readily exchange genetic information is through transposition. Transposition describes the translocation of a discrete segment of DNA (the transposable element or transposon) from a donor site to non-homologous target sites. Transposable elements encode the machinery required to execute such rearrangements in addition to other determinants such as antibiotic resistance genes and genes for virulence factors. In general, transposition is an infrequent event probably because of its capacity for deleterious effects in the host. Usually, a transposon is translocated onto a plasmid upon conjugation. This may be followed by the integration of the transposon into the chromosome. For many transposons, however, plasmids rather than the bacterial chromosome appear to be the preferred target (Craig 2014).

32. Although there is a constant and frequent flux of DNA in *E. coli*, especially through plasmids, chromosomal insertion and deletion events are not random. Most gene acquisitions and losses happen in the exact same locations (‘hotspots’) leaving the core genome largely unchanged (Tenaillon et al. 2010).

4.3 Pathology of *E. coli*

33. Pathogenic *E. coli* are classed in different pathotypes, depending on the nature of their pathogenicity, such as host range or tissue tropism. These pathotypes are thought to be unified by specific combinations of virulence traits (Russo & Johnson 2000). The most extensive research of various pathotypes has focused on human pathogenic *E. coli*. There were several main *E. coli* pathotypes identified including: enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC/Shigella), enteroaggregative (EAEC) and extraintestinal-pathogenic (ExPEC, amongst which the uropathogenic, UPEC, is the most common) (Salyers 2011). To discuss every pathotype and representatives of each pathotype is well beyond the scope of this document, the remainder of this RARMP will focus on a specific ExPEC pathotype, the avian pathogenic *E. coli* (Donnenberg 2013).

4.4 Avian pathogenic *E. coli*

34. The parent organism of the GMO in this licence application is *E. coli* O78, a member of the avian pathogenic *E. coli* (APEC) pathotype. APEC infections occur exclusively in birds (Barnes & Gross 1997; Lister & Barrow 2008).

35. APEC are the causal agent for colibacillosis, a localised or systemic infection in birds which includes a range of infections such as coli septicaemia, haemorrhagic septicaemia, swollen head syndrome, air sac disease, peritonitis and many more (Barnes & Gross

1997; Dho-Moulin & Fairbrother 1999; Lister & Barrow 2008). Most APEC are also classed as ExPEC and share characteristics with mammalian ExPEC.

36. APEC cause colibacillosis in all birds, but it is of particular concern for birds used in the poultry industry as symptoms of avian colibacillosis include respiratory distress, reduced appetite, poor growth, and severe cases can lead to septicaemia and death (Barnes & Gross 1997; Dho-Moulin & Fairbrother 1999; Lister & Barrow 2008). The range of disease manifestations depends on a number of factors such as the APEC serotype, the virulence factors carried by the bacteria, and host and predisposing factors (Barnes & Gross 1997; Dziva & Stevens 2008).
37. Colibacillosis in poultry is often observed as a secondary infection, the idea that avian colibacillosis is an opportunistic infection is widely accepted (Swayne 2013). This can occur when birds are infected with another organism, which compromises the immune system. Poultry are also more susceptible to APEC infection as a result of environmental stresses (such as poor living conditions, and inadequate food and/or water) (Barnes & Gross 1997; Dho-Moulin & Fairbrother 1999; Lister & Barrow 2008). Thus, colibacillosis can be controlled through maintenance of high standards of health and wellbeing within a flock (Dho-Moulin & Fairbrother 1999; Lister & Barrow 2008).
38. The time between infection and onset of clinical symptoms varies, depending on the specific type of disease caused by APEC. Incubation period is generally short, between 1 and 3 days and the onset of clinical disease symptoms can be observed 5-7 days after infection with a primary agent such as infectious bronchitis virus (Swayne 2013).
39. Spread of APEC infections may occur through a number of routes. APECs, although causing disease outside the intestinal tract, can reside in the intestine. As such they can be shed by an infected animal in the faeces (Lister & Barrow 2008). Oral and respiratory routes are known to cause infection, through exposure to contaminated litter, water or feed (Dho-Moulin & Fairbrother 1999; Dziva & Stevens 2008). APEC can survive for extended periods in dry litter, although it is known that wetting of contaminated litter can reduce the incidence of infections (Lister & Barrow 2008). Inhalation of dust from contaminated litter can lead to systemic APEC infection in other poultry (Dziva & Stevens 2008). Shedding of the bacteria in faeces can also lead to infection of eggs (Barnes & Gross 1997; Dziva & Stevens 2008; Giovanardi et al. 2005; Lister & Barrow 2008). Contact between birds can also spread APEC infections (Dho-Moulin & Fairbrother 1999).
40. APEC infections are common worldwide. The most common serotypes are O1, O2, O35 and O78 (Dho-Moulin & Fairbrother 1999; Ike et al. 1990; Jeong et al. 2012). However, the serotype prevalence varies between countries. Serotype prevalence data is not comprehensive. A survey from 1985-1986 showed that O78 was the most common serotype in Australia (Murray 1987).
41. APEC carry a large range of virulence factors, many of which are carried on extrachromosomal plasmids (Dho-Moulin & Fairbrother 1999; Dziva & Stevens 2008; Ginns et al. 2000; Jeong et al. 2012; Mellata et al. 2010; Rodriguez-Siek et al. 2005b). These plasmids are essential for APEC virulence (Ginns et al. 2000; Mellata et al. 2010). As discussed above, APEC cause a range of diseases, and this is thought to be related to the virulence factors carried by subpathotypes. One study suggested that APEC isolates are likely to share a common set of virulence factors, many of which are found on an extrachromosomal plasmid (Rodriguez-Siek et al. 2005b).
42. APEC are known to share virulence attributes with other ExPEC. There is some evidence for the zoonotic potential of APECs, including from phylogenetic relationships, genome sequences, gene expression profiles, shared virulence traits and results from animal

models (Bauchart et al. 2010; Ewers et al. 2009; Johnson et al. 2007; Moulin-Schouleur et al. 2007; Rodriguez-Siek et al. 2005a; Tivendale et al. 2010).

43. Caya et al. (1999) found very little evidence for the zoonotic potential of *E. coli* strains isolated from chickens, and Kaper (2005) states that the APEC 'do not seem to have a close counterpart in human disease'. However, some human and avian ExPEC share some virulence genes (Manges and Johnson 2012).

44. To date, there has been no confirmed incidence where an avian ExPEC strain caused a disease in human. There has also been no report of any subclinical infection in a human caused by an APEC. However, some human ExPEC can cause disease in chicken models for colibacillosis and some avian ExPEC can cause infection in animal models for human infection (Zhao et al 2009; Tivendale et al 2010). A limited pathogenic potential may be related to large plasmids found in APEC (Mellata et al. 2010). Growing evidence suggests that transfer of APEC plasmids could be a source of virulence genes for other ExPEC strains leading to disease (Johnson et al 2012; Olsen et al 2012).

45. A scientific assessment of the safety of poultry meat in Australia was conducted by Food Standards Australia and New Zealand (FSANZ) (2005). This concluded that:

> According to available data, there are no significant public health and safety risks resulting from pathogenic *E. coli* in poultry or poultry meat products in Australia. Although human pathogenic strains such as enterohaemorrhagic *E. coli* (EHEC) have infrequently been isolated from poultry internationally, there has been no documented case of food-borne illness due to *E. coli* associated with consumption of poultry meat in Australia.

46. In addition, the Foodborne Diseases Working Group also concluded that *Campylobacter* and *Salmonella* are the major agents from poultry that cause disease in people (Foodborne Disease Working Party for the Communicable Diseases Network Australia and New Zealand 1997), and that *E. coli* is not a pathogen of concern.

4.5 Susceptibility to antibiotics and other chemical agents

47. APEC are susceptible to many antibiotics (Barnes & Gross 1997; Dho-Moulin & Fairbrother 1999; Lister & Barrow 2008), but some isolates can show resistance to one or more antibiotics, especially if the antibiotics have been widely used in the poultry industry over a long period (Barnes & Gross 1997; Dho-Moulin & Fairbrother 1999; Wang et al. 2010).

48. *E.coli*, including APEC, are susceptible to many commonly used disinfectants, including chlorine and bleach (Oie et al. 1999; Public Health Agency of Canada 2012).

Section 5 The GM vaccine – nature and effect of the genetic modification

49. The vaccine contains live genetically modified *Escherichia coli*. The parent strain was isolated from a clinical case of colibacillosis in the United Kingdom. The parent organism was serotyped as an O78 strain. The GM *E. coli* vaccine has been produced by deleting 100 base pairs (bp) of the essential *aroA* gene. The applicant proposed to use the vaccine in chickens only.

50. The *aroA* gene encodes the 3-phosphoenolpyruvylshikimate-5-phosphate synthetase (EPSP), a key enzyme of the aromatic amino acid biosynthetic pathway (Duncan & Coggins 1986). Inactivation of EPSP activity through deletion results in an auxotrophic organism, an organism that has lost the ability to synthesize certain substances required for its growth and metabolism, and it cannot grow in the absence of these amino acids.

51. The GM *E.coli* is attenuated due to this inability to synthesize aromatic amino acids when compared to the wild type organism.

5.1 Introduced modification and its associated effects

52. PCR was used to amplify two segments from the 5′ and 3′ regions of the *aroA* gene. These were then ligated into a shuttle plasmid. The resulting *aroA* sequence in the shuttle plasmid was missing 100 bp from the central region of the wild type gene. Two stop codons and two restriction endonuclease sites were also introduced into the *aroA* gene with the 100 bp deletion (*aroA*-).

53. The *aroA*- gene was then excised from the shuttle plasmid and ligated into a second plasmid vector. This plasmid carrying the modified *aroA* gene was then transformed into an intermediate bacterial donor strain. This donor strain was mated with the parent organism (*E. coli* O78) and conjugation between the strains allowed the uptake of the plasmid carrying the *aroA*- gene into the *E. coli* O78.

54. Homologous recombination between the wild-type *aroA* gene in the chromosome of the parental strain and *aroA*- gene carried by the vector produced a modified APEC O78 strain carrying the modified *aroA*-.

55. The 100 bp deletion of the *aroA* gene as well as the introduced stop codons ensure that a functional *aroA* protein cannot be made by the GMO. The restriction endonuclease sites were introduced to facilitate identification of the GMO. No additional sequences have been introduced into, or deleted from, the parent organism.

5.2 Characterisation of the GMO

5.2.1 Genotype and phenotype stability, molecular characterisation

56. The genome of the GM *E. coli* vaccine strain has not been fully sequenced. Unintentional changes through the introduction of *aroA*- construct (carrying the two stop codons and two restriction sites) are possible, no additional genomic sequences were introduced into the genome. No analysis of metabolites was performed with the GMO.

57. The genetic stability of the GM *E. coli* vaccine was studied by performing five backpassages in chicken. During the backpassages, no clinical signs of disease were observed and the presence of GM *E. coli* was confirmed by PCR. No genotypic or phenotypic reversion to the pathogenic *E. coli* strain was detected.

58. The GM *E. coli* was screened for 54 genotypic traits that are associated with virulence factors. Four virulence associated genes were detected and are located on the chromosome.

59. BLASTn analysis of the complementary strand of the novel *aroA*- construct did not reveal any homologies to known genes (BLASTn at http://blast.ncbi.nlm.nih.gov/Blast.cgi).

5.2.2 Studies conducted by the applicant on clearance, shedding and ability to spread of the GMO

60. A number of studies have been conducted by the applicant to address clearance, shedding and spreading of the GMO. The results of these studies were included in the licence application.

61. In one study, 50 one-day-old chickens were administered the GM vaccine using coarse spray [at a dose of 2.2×10^8 colony forming units (CFUs)], and tested for the presence of the GM *E. coli* in internal tissues at days 4, 8, 11, 15 and 22. The GMO was only recovered from one bird at day 4 from heart and liver tissue. Litter samples were also taken at each time point. The GMO was only recovered at days 4 and 8. A contact control group, consisting of 25 chickens, was also tested for presence of the GM *E. coli*. No GMO was recovered at any time for this group.

62. In another study, 51 one-day-old chickens were administered the GM vaccine using eye drops (at a dose of $6.4x10^9$ CFUs); they were housed together with 25 unvaccinated birds. Internal tissues, including hearts and livers, of the birds were tested for the presence of the GMO at days 4, 7, 11, 14 and 21. The GMO was recovered from the internal tissues (heart and liver) of two birds at day 4. The GMO was not recovered from the internal tissues of any other vaccinated bird at any time. No GMO was recovered from internal organs of any bird of the unvaccinated control group at the end of the study.
63. In the latter experiment, nasal and cloacal samples (swabs) were also taken each time. The GMO was detected in cloacal samples up to day 14 in vaccinated birds, and up to day 11 in non-vaccinated birds. Samples were also taken from drinking water, feed and litter at each time point. The GMO was recovered up to day 7 in drinking water, and up to day 21 (end of study) in feed and litter.
64. The presence of GMO in the cloacal samples is thought to be due to the route of administration in this study. In eye drop administration, the GMO can pass from the nasal cavity to the oral cavity, and then swallowed into the gut. The applicant states that its presence in the cloacal swabs at day 14 in the vaccinated birds may indicate limited replication in the gut. This is proposed to occur due to the presence of aromatic amino acids in the gut from food, or from other bacteria able to synthesize aromatic amino acids.
65. The presence of the GMO in the cloacal samples of non-vaccinated birds indicates the GMO can be spread from vaccinated to non-vaccinated birds. The applicant suggests this has occurred through exposure of non-vaccinated birds to contaminated faeces and the contaminated environment (such as water and feed). No GMO could be isolated from tissues of the non-vaccinated birds indicating limited replication.
66. In a third study, 40 one-day-old chickens were exposed to the GMO, half by eye drop (at a dose of $4.9x10^9$ CFUs) and half by coarse spray (at a dose of $7.5x10^9$ CFUs). Cloacal samples were taken at days 1, 7, 14, 21, 28, 35 and 42. Environmental samples were also taken at the same time points from water, feed and litter. The GMO was detected in cloacal samples from day 1 to day 28 in birds exposed by eye drop and coarse spray. Positive results decreased over time for both the cloacal and environmental samples. At day 35, all birds tested negative for presence of the GMO. Environmental samples were positive for the GMO to day 35; they peaked at day 7.

5.2.3 Studies conducted by the applicant on environmental persistence of the GMO

67. In one study, chicken litter was inoculated with the GMO, or with wild type *E. coli* serotype O78 and kept under standard laboratory conditions (ie at 20°C in a sterile environment). The number of CFUs recovered declined over 24 hours, after which no GMO could be recovered. Wild-type *E. coli* was recovered at 24 hours, but not recovered at 48 hours.
68. In another study, feed, water and litter were inoculated with the GMO, kept under standard laboratory conditions (ie at 20°C in a sterile environment) and samples were taken up to 42 days post-inoculation. In litter, there was a rapid decrease in GMO titre, with no recovery of the GMO by day 7. In feed, the GMO was recovered throughout the experiment, up to and including day 42, with a $3\log_{10}$ reduction by day 42. In water, the GMO was recovered throughout the experiment, up to and including day 42, with a $2\log_{10}$ reduction by day 42. These results indicate that the GM *E. coli* does not replicate or the rate of cell death is higher than the rate of replication.
69. One study looked at the presence of GM vaccine isolated from litter, water and feed at distinct time points. One day old chickens were vaccinated with the GM vaccine and the persistence of the GMO was studied. GM *E. coli* could be detected up to 35 days post vaccination with no GMO detectable 42 days post vaccination.

5.2.4 Studies conducted by the applicant on potential for horizontal gene transfer and potential for reversion of the GMO to a pathogenic form

70. In one study, one-day-old chickens were inoculated with the GMO (at doses of $3.1x10^7$, $8.3x10^6$ and $1.4x10^7$ CFUs, respectively), and tissue samples were analysed at days 4, 8 and 21 post-inoculation. The study protocol intended to recover GMOs from inoculated birds for subsequent backpassage as part of the reversion to virulence study. However, no GMOs were recovered from any birds at any time points and they did not show any symptoms associated with colibacillosis.

71. In another study, 5 one-day-old chickens were inoculated with the GMO (at a dose of $2.2x10^9$ CFUs). After four days, liver tissues were harvested. GMO was recovered from three of the five birds. Swabs positive for the GMO from these three birds were pooled and used to inoculate another 5 birds. After four days, tissues were harvested. No GMO was recovered from any birds. A further 10 birds were inoculated with the swab samples positive for the GMO from the first five birds. After four days, liver tissues were harvested. No GMO was recovered from these birds and during the study there were no signs of clinical disease in any of the birds.

72. In a third study, 10 one-day-old chickens were inoculated with the GMO (at a dose of $1.2x10^7$ CFUs). After seven days, liver tissues were harvested. GMO was recovered from four of the ten birds. Swabs positive for the GMO from these four birds were pooled and used to inoculate another 10 birds. After seven days, tissues were harvested. GMO was recovered from one of the ten birds. Swabs positive for the GMO from this bird was used to inoculate another 10 birds. After seven days, tissues were harvested. No GMO was recovered from any birds and during the study there were no signs of clinical disease in any of the birds.

73. In conclusion, the results from these three studies indicate that the GMO did not revert to virulence and that the birds were not harmed by the GM vaccine.

5.2.5 Studies conducted by the applicant of effects of the vaccine on non-target animals

74. In one study, 15 three week old piglets were inoculated with the GMO (at a dose of $3.6x10^8$ CFUs). All animals were observed daily for signs of clinical disease. Over the period of the study, there were no adverse effects to the animals that were attributable to the GMO. At the end of the study (6 weeks), animals were necropsied. The studied tissues (lung, heart, liver and spleen) showed no visible lesions. Additionally, no GMO was recovered from these tissues.

75. In another study, two groups of 8 mice were inoculated with the GMO, one intraperitoneally (at a dose of $1.5x10^7$ CFUs) and one intracerebrally (IC) (at a dose of $1.0x10^6$ CFUs). Mice were observed daily for 7 days. One mouse died at the time of IC inoculation. This is proposed to be due to trauma from administration of the GMO. All other animals survived for the whole period of the study with no clinical signs of a disease.

5.2.6 Effect of the GMO on humans

76. No clinical trials investigating the effect of the Poulvac *E. coli* ® vaccine on human volunteers have been conducted.

77. The parent organism *E. coli* EC34195 is considered not to be a human pathogen (Caya et al. 1999; Kaper 2005).

78. APECs tend to be less toxigenic compared to mammalian pathogenic *E. coli*. This could be due to the lack of toxin production or the toxins produced are not readily detectable by

mammalian toxicology screens (Blanco et al. 1997; Janssen et al. 2001; Mellata et al. 2001).

79. A pharmacovigilence report produced by Pfizer Inc looked at the number of doses of Poulvac *E. coli*® sold and the number of adverse event cases reported in the USA over a 4 year period. No confirmed cases of adverse reactions in humans or the environment were found.
80. FSANZ could not identify *E. coli* as a pathogen of concern for meat from poultry. Zoetis would require an approval from the APVMA to supply the GM vaccine. As part of the assessment of the GM vaccine, the APVMA would consider the risk posed by the presence of residual vaccine in meat and eggs of chickens.
81. On commercial poultry farms, meat chickens (broilers) are raised in batches. When they reach market weight they are transported to processing plants. Broilers are not expected to be exposed to a second vaccination with the GM vaccine as they are usually sent off for processing between 6 and 7 weeks of age. No GM vaccine could be detected in chickens that were older than 35 days in trials on poultry farms.
82. There is a potential for limited replication of the GMO in eggs but it is unknown to what level this might occur.
83. Eggs for human consumption are routinely washed prior to packaging. They are inspected visually and eggs identified as cracked and/or dirty must not be sold. They must be clean with no visible cracks, faeces, soil or other foreign matter (Australian egg corporation).
84. The eggs must be derived only from healthy stock and, when medication has been given to a flock, eggs will not be sold during the recommended withholding period.

5.2.7 Antibiotic susceptibility of the GMO

85. Data has been supplied by the applicant that lists the antibiotic sensitivities of the parental strain, and the GMO. They are sensitive to many commercially available antibiotics.

Section 6 The receiving environment

6.1 Relevant environmental factors

86. If a licence were issued by the Regulator as well as authorisation given by the other relevant regulators, the GM chicken vaccine may be used on any commercial chicken farm in Australia.
87. The primary environment receiving the GM *E. coli* vaccine would be the upper gastrointestinal (GI) tract of the vaccinated birds.
88. The extended receiving environment is the poultry farms on which the GM chicken vaccine is administered, particularly any sheds or similar areas where the GM chicken vaccine would be administered, as well as any run-off areas and those areas where chicken carcasses, litter etc would be composted or disposed of (buried or incinerated). In addition, areas onto which the GM chicken vaccine might be spilled during import, transport or storage would also come into contact with the GM *E. coli*.
89. In Australian poultry farms, chickens are mainly grown for their meat (broilers) and for their eggs (layers). Breeds used for egg laying are different to those used for meat production. Worldwide, most commercial chickens originate from the same parent breeds that were developed by specialised breeding companies.
90. Chicken farms are present all over Australia, with the majority of chicken grow-out farms (meat production) located within 100km of poultry processing plants (Australian chicken meat federation). Poultry processing plants, where mature broilers are sent for processing,

have developed near urban centres due to the proximity of markets and consumers. The distribution of layer poultry farms (egg production) in Australia is determined by the population density and by the availability of feed ingredients, mainly cereal grains (Australian egg corporation). In 2011, over 392 million dozen eggs were produced for human consumption; in 2007 an estimated 460.3 million broilers were grown for commercial meat production (Poultry hub).

91. Chickens farms generally adopt one (or more) of the three main production systems: free range, barn and cage system. Free range egg and meat production make up 10 to 15% of the total market. Meat birds are kept in large open sheds or barns or are free range. The majority of layers are kept in cages (about 55%) with the rest either free range (35%) or in barns (9%).
92. The GM chicken vaccine would be applied, and unused vaccine or waste material would be disposed according to manufacturer's instructions and to conditions, if any, imposed by the APVMA.
93. Other diseases that are controlled by vaccination in Australia include: Newcastle disease, fowl pox, chronic respiratory disease and egg drop syndrome 76.

6.2 Presence of related bacterial species in the receiving environment

94. *E. coli* is a commensal of mammals, birds, reptiles and amphibians, and is also present in the wider environment. The preferred niche of *E. coli* is the gastro intestinal (GI) tract of larger animals due to longer GI and time of passage through the GI, and available nutrients (Donnenberg 2013).
95. Enterobacteria, including *E. coli,* are found in poultry gastro intestinal tract (GI) as part of the native flora. It is recognised that most poultry already carry wild type *E. coli* O78 as part of this native flora.

6.3 Presence of the *aroA* gene in the environment

96. The *aroA* gene is found in a wide range of bacterial species in the environment, including *E.coli*.
97. Other bacterial species with an *aroA* gene or closely related homologue include *Chlamydia pneumonia*, *Deinococcus radiodurans*, *Fusobacterium nucleatum* and *Mycobacterium tuberculosis* (source BLASTn at http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Section 7 Relevant Australian and international approvals

7.1 Australian approvals

98. This GM *E. coli* vaccine has not been previously approved in Australia.

7.1.1 Approvals by other government agencies

99. This GM chicken vaccine has not been approved by other Regulators in Australia.

7.2 International approvals of GM Poulvac *E. coli*® vaccine

100. The GM vaccine has been registered for commercial sale (under trade name Poulvac *E.coli*®) in several countries and territories, including the United States, Europe, Brazil and Philippines.
101. Poulvac *E.coli* ® was registered in the United States in 2006 and is currently used in poultry farms with over 5 billion doses administered worldwide.

Chapter 2 Risk assessment

Section 1 Introduction

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

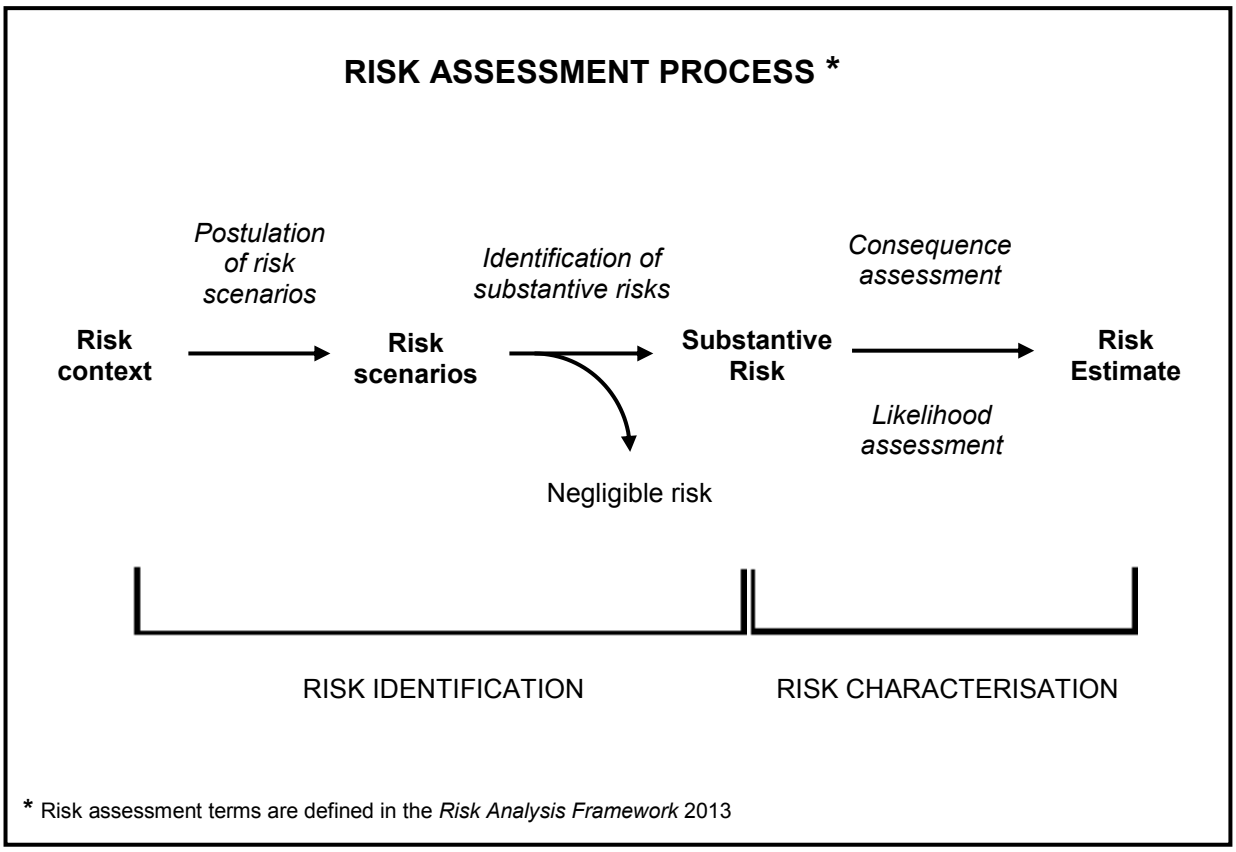

Figure 2. The risk assessment process

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

104. Each risk scenario is evaluated to identify those risks that warrant detailed characterisation. A risk is only identified for further assessment when a risk scenario indicates the possibility of substantive risk. Pathways that do not lead to harm, or could not plausibly occur, do not advance in the risk assessment process.

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

106. Substantive risks (*i.e.* those identified for further assessment) are characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The level of risk is then estimated from a combination of the Consequence and Likelihood assessments. The level of risk, together with analysis of interactions between potential risks, is used to evaluate these risks to determine if risk treatment measures are required.

Section 2 Risk Identification

107. Postulated risk scenarios are comprised of three components (Figure 3):

i. The source of potential harm (risk source)

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

iii. Potential harm to an object of value (people or the environment).

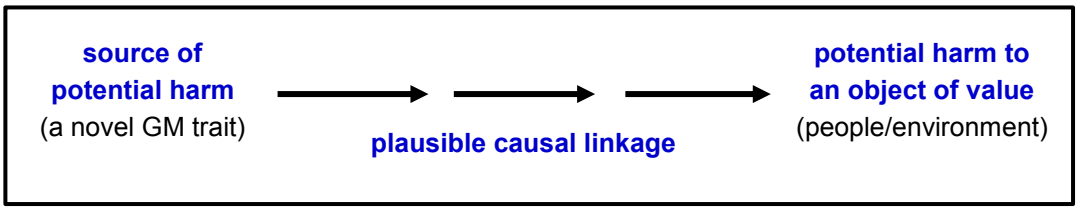

Figure 3. Risk scenario

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

- the proposed dealings, which are to import, transport or dispose of the GMOs and the possession (including storage), supply and use of the GMOs in the course of any of these dealings
- the proposed controls and relevant practices;
- characteristics of the parent organism;
- different routes of exposure to the GMO;
- potential exposure of genes and gene products to the environment.

2.1 Risk source

2.1.1 The GMO

109. A source of the potential harm can be the traits associated with the GMO or some unintended effects arising from modifying the genetic element.

110. As discussed in Chapter 1, the GM *E. coli* has been modified by the deletion of 100 bp of the *aroA* gene, introduction of two stop codons and two restriction enzyme cutting sites at the same gene.

111. Theoretically there could be changes to metabolites of the GMO that were detrimental to the health and safety of people or the environment. However, if there were changes to the metabolites with the potential to increase the level of harm, they would have appeared either in the various studies assessing the efficacy of the GM vaccine or during the extensive commercial releases in other countries.

112. There were no additional genetic elements intentionally introduced in the GM *E. coli*. Therefore, the risk of unintended effects resulting from the process of genetic

modification or the modified gene is negligible and will not be considered further for this application.

2.1.2 Inherited traits from the parental E. coli strain

113. Another source of potential harm relates to certain traits of the parental *E. coli* O78, which was used to generate the GMO.

114. The presence of four virulence associated genes in GM E. coli O78 was demonstrated in one study. They were found to be located on the chromosome and are therefore less likely to be transferred to other pathogenic bacteria. Additionally, the GMO is replication deficient in the absence of free aromatic amino acids, which is expected to further reduce the potential to share genetic information with other bacteria.

115. The GMO is a registered vaccine in Europe, USA and many other countries and territories. The organism has been assessed by a number of regulatory agencies, including the Directorate-General for Health and Consumers of the European Commission and the USDA.

116. Therefore, the risk associated with the virulence factors of GM *E. coli* are negligible and will not be considered further.

2.1.3 Potential off-target effects

117. Several studies were conducted to investigate potential off-target effects of the GM vaccine. Piglets and mice were challenged with the GM *E. coli* vaccine and no clinical symptoms associated with the vaccine could be observed. The studies concluded that the GM E. coli vaccine was safe when administered to piglets and mice.

118. Based on all the available data, the risk of this GM *E. coli* to the health and safety of mammals is negligible as a disease causing agent. Therefore, the risks from the GMO to mammals (excluding humans) will not be considered further.

2.1.4 Potential harm to poultry

119. Several studies, including a large scale field study, were conducted by the applicant to investigate the safety of the GM vaccine under controlled and field conditions (poultry farms). When comparing vaccinated and control groups, vaccinated chickens and turkeys outperformed the non-vaccinated group in both clinical and performance parameters. No detrimental effects of the GM vaccine on chicken were observed.

120. To date, five billion doses of Poulvac *E. coli*® vaccine have been administered worldwide without any confirmed reports of adverse effects.

121. APVMA will assess the efficacy of the vaccine and the potential risks from the GMO to poultry.

2.2 Plausible causal linkage

122. The following factors are taken into account when postulating plausible causal pathways to potential harm that arise from the proposed dealings relating to import, storage, transport and disposal:

- different pathways of exposure of people and the environment to the GMO
- reversion of the GM *E. coli* to wild type
- gene transfer by horizontal gene transfer (HGT), including acquiring tolerance to antibiotics
- the environment at the site(s) of release
- spread and persistence of the GMO, including

- establishment
- reproduction
- dispersal.

2.2.1 Horizontal Gene Transfer

123. During storage, transport or disposal of the vaccine the GM *E. coli* could come into contact with other *E. coli* or related species.

124. Studies have shown that the GM *E. coli* is susceptible to many commercially available antibiotics. In case the organism acquires resistance to an antibiotic through horizontal gene transfer, other antibiotics would be available to which the GM *E. coli* is susceptible. A large scale study in Morocco commissioned by the applicant has demonstrated that in bird houses with vaccinated chickens the number of antibiotic treatments required was lower than for the control group. The risk associated with HGT of antibiotic resistance genes is negligible and will not be considered further.

125. HGT from GM *E. coli* to other non-GM *E. coli* or related species is mainly limited to plasmids. The four virulence associated genes as well as the *aroA* deletion are located on the chromosome and would be transferred to other *E. coli* or related species infrequently. As *E. coli* O78 was isolated in Australia previously, it is likely that these virulence factors are already present in the environment. HGT of the *aroA* deletion to other *E. coli* or related species would be detrimental for the recipient as it would no longer be able to produce aromatic amino acids. Therefore, the risk associated with HGT from GM *E. coli* to non-GM *E. coli* or related bacteria is negligible and will not be considered further.

2.2.2 Spread and persistence

126. As discussed in Chapter 1, several studies have addressed the persistence of the GM *E. coli* under various conditions. '*In vitro*' testing showed that the organism can survive for at least 42 days in water, feed and commercial litter with decreasing numbers of GM *E. coli* over time. When tested under conditions similar to a commercial poultry rearing environment (sterilized litter from a poultry farm) or on actual poultry farms, the GMO was not found to persist for more than 35 days post-vaccination.

127. When vaccinated and non-vaccinated chickens were kept together, GM *E. coli* could be detected in faeces of non-vaccinated birds up to 14 days post vaccination. The study concluded that the spreading occurs in the first days post vaccination through contact with faeces from vaccinated birds. Because of limited replication potential, no GMO could be detected after that period.

128. Poultry production facilities apply strict biosecurity measures and standardised farm practices. They include that chicken litter is treated appropriately (composted, incinerated or buried) and dead birds are removed daily and sent off to rendering plants. Chicken farms deal with the potential threat of existing bacterial and viral diseases in a way that the consumer is not affected through any of the products and by-products of chicken production (meat, eggs, litter and dead birds). Chicken farms are fenced and netted to protect chickens from wild birds, predators and other wild animals and to minimize the access of wild birds to feed and water.

129. Therefore, the risk associated with spread and persistence of the GMO is negligible and will not be considered further.

2.3 Potential harm

130. Potential harms from GM *E. coli* vaccine include:

- harm to the health of people or desirable organisms, including toxicity/allergenicity

- reduced establishment of desirable organisms, including having an advantage in comparison to related bacteria
- reduced biodiversity.

131. The potential harm to the health of people, the reduced establishment of desirable organisms and the increased number of feral or pest birds will be discussed further in the risk scenarios below.

2.4 Postulated risk scenarios

132. Three risk scenarios were postulated, evaluated and listed in Table 1. In the context of the proposed dealings and considering both the short and long term, these risk scenarios do not give rise to any substantive risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment. Details of the evaluation of the scenario are provided later in this section.

Table 1 Summary of risk scenarios from the proposed dealings with GM *E. coli* vaccine

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk?	Reason
1	GM *E. coli* O78 *aroA*-	Exposure of people to the GMO through: - Accidental spills - Fertilizer (chicken manure) ⬇ Infection of the host	Disease effects in people	No	• People have to adhere to the general guidelines for handling veterinary products. • The parental *E. coli* strain O78 is not currently considered a human pathogen. • There is a history of safe use of the GM chicken vaccine. • There is limited shedding of the GMO into the environment. • GM *E. coli* O78 has reduced spread and persistence in the environment compared to the parental strain.
2	GM *E. coli* O78 *aroA*-	Reversion of GMO to virulent strain ⬇ Exposure of native wild birds to virulent APEC O78 ⬇ Development of colibacillosis in wild birds	Reduced number of desired wild birds	No	• There is a history of safe use of the GM chicken vaccine (reversion to virulence has not been observed). • Colibacillosis is considered a secondary disease. • Wild type *E. coli* O78 is likely to be present in the environment.
3	GM *E. coli* O78 *aroA*-	Exposure of feral or pest birds to the GMO ⬇ Immunization of feral or pest birds	Increased number of feral or pest birds	No	• There is limited shedding of the GMO into the environment. • There is limited access of feral birds to the GMO. Use of the vaccine as per prescription would minimise the likelihood of exposure of GM *E. coli* to feral birds. • GM *E. coli* O78 is attenuated and, therefore, its spread and persistence in the environment is reduced compared to the parental strain. • Feral or pest birds are unlikely to be challenged with a sufficiently large dose of attenuated GM *E. coli* to induce an immune response. • There is a history of safe use of the GM chicken vaccine.

2.4.1 Risk scenario 1

Risk source	Causal pathway	Potential harm
GM *E. coli* O78 *aroA*-	Exposure of people to the GMO through: - Accidental spills - Fertilizer (chicken manure) ⬇ Infection of the host	Disease effects in people

Risk source

133. The source of the potential harm for this risk scenario is the GM *E. coli* O78 *aroA*-.

Causal pathway

134. People could be exposed to the GMO in various ways, including: accidental spills may occur during transport, possession (storage) and disposal; handling of chicken manure containing GMOs may lead to exposure.

Potential harm

135. That the GMO causes disease in humans.

Exposure of people to GMO through accidental spills

136. People could potentially get exposed to a high dose of the GM vaccine after an accidental spill if the container holding the lyophilised powder broke or while preparing the vaccine for administering it to chickens. To date, there has been no confirmed case of an APEC causing infection (clinical or subclinical) in humans. It is, therefore, not likely to cause clinical disease symptoms in people exposed to the bacteria. Should there be such an event in the future, the outcome of this event would be no different to that when people were exposed to the parent organism. There is no information available about exposure to lyophilised *E. coli* O78 but the effects of inhalation endotoxins from *E. coli* have been studied (Michel et al 1997; Loh et al 2006; Doyen et al 2010; Michel O 2000). Based on the findings in these studies it is not expected that the outcome of inhaling the GMO is different to inhaling the parent organism.

137. There is the potential that humans are exposed to the GM *E. coli* when preparing or administering the vaccine. A pharmacovigilance study from the USA reported that around 1 billion doses of Poulvac *E. coli*® vaccine were administered without any confirmed harm to humans.

138. The GM vaccine is a registered veterinary vaccine in Europe, USA and many other countries and territories. The organism has been assessed and regarded as safe by a number of regulatory agencies including the Directorate-General for Health and Consumers of the European Commission and the USDA.

139. Non-target studies in piglets and mice concluded that the piglets and mice challenged with the GM *E. coli* vaccine did not show any clinical symptoms associated with the vaccine.

Exposure to the GMO through fertilizer (chicken manure) and dead chickens

140. Chicken manure is a widely available and commonly used as fertilizer. Studies on environmental persistence of the GMO have demonstrated that no GMO was present in chicken litter 42 days post vaccination.

141. Meat chicken sheds operate as closed systems with little or no water movement from sheds to ground water or to drainage lines. Shed floors are swept clean after the broilers

are sent off for processing and litter is composted on a concrete slab or other suitably impermeable material and covered by a roof. This happens either on site or is performed by a suitable contractor. These measures are designed to prevent contamination of ground or surface waters or the surrounding area and to achieve the necessary temperatures for destruction of pathogenic bacteria and viruses.

142. The standard management practice for the treatment of dead birds requires daily collection from the shed and removal from the farm for rendering. If farms do not have ready access to a rendering plant, the next preferred method of disposal is composting. It is important to understand that chicken carcases harbour many, potentially pathogenic, bacteria and viruses. Current rendering techniques ensure that humans and the environment are not exposed to any potential pathogens.

143. Other methods of disposal include burial or incineration. The relevant local government authorities are consulted on the most appropriate and allowable carcass disposal method.

144. For egg laying chickens, cleaning and sanitising of poultry houses is carried out between flocks for single aged houses or at the batch turn around (at least once yearly) for multi-aged houses. The litter of layers is treated in the same way as the litter from broilers.

145. Chickens harbour many microorganisms, including (non-GM) *E. coli*. Some of these microorganisms are pathogenic in chickens, other animals and humans. Examples include *Campylobacter* and the highly pathogenic *Salmonella*. Therefore, biosecurity measures in poultry farming are very important to the industry, the public and our environment; they are integral to current industry practices. These biosecurity measures are effective on pathogens that are known to cause disease in humans and other organisms. They are considered appropriate and effective on the GM *E. coli* which has not been demonstrated to cause harm to humans or the environment.

Conclusion

146. Risk scenario 1 is considered to be a negligible risk due to the causal pathway and the potential harm being highly unlikely to eventuate. Based on the available data, the GM vaccine is unlikely to be a disease causing agent or as a reservoir for virulence associated genes. Therefore, this risk is not considered to be a substantive risk that warrants further detailed assessment.

2.4.2 Risk scenario 2

Risk source	Causal pathway	Potential harm
GM *E. coli* O78 *aroA*-	Reversion of GMO to virulent strain ⬇ Exposure of native wild birds to virulent APEC O78 ⬇ Development of colibacillosis in wild birds	Reduced number of desired wild birds

Risk source

147. The source of the potential harm for this risk scenario is the GM *E. coli* O78 *aroA*-.

Causal pathway

148. During disposal chickens and other birds could be exposed to the GM vaccine.

149. The GM *E. coli* could revert to virulence by acquiring a functional *aroA* gene or gene homologue. This would allow the bacteria to replicate, spread and persist in the environment. Wild birds exposed to this infectious form develop colibacillosis.

150. Reversion to virulence relies on acquiring a functional *aroA* gene (or gene homologue) from other *E. coli* bacteria or related bacterial species.

151. Several studies addressed the potential revision to a virulent form of the *E. coli* O78 strain. Successive backpassage experiments were performed where chickens were initially inoculated with the *E. coli aroA*- master seed and the *E. coli* recovered from these chickens was used to inoculate other chickens. The birds were observed for clinical signs and euthanized at the end of the studies. Autopsies were performed to identify symptoms typically associated with colibacillosis. No unfavourable events were recorded and the studies concluded that the *E. coli aroA*- master seed did not revert to virulence.

152. GM *E. coli* is unable to grow in the absence of aromatic amino acids. The partial deletion of the *aroA* gene leaves the GMO unable to produce these aromatic amino acids and access to free amino acids in the environment is limited. The *aroA* gene is located on the chromosome, homologous recombination through transfer of *aroA* from a compatible organisms is unlikely. To facilitate the transfer of genetic elements from one cell to another *E. coli* needs to be in an active, reproductive cell cycle. GM *E. coli aroA*- has limited replication potential and hence transfer of genetic elements through transduction or transformation is unlikely. Secondly, *E. coli* and other enterobacteria (the most likely source of *aroA* gene) reside in the caecum of birds. As GM *E. coli* is growth deficient, the total number of GMOs will decline as they move down the GI tract of the birds. Thus, only a limited number of GM *E. coli* will reach the caecum limiting the exposure to other enterobacteria. Thirdly, the conditions in the caecum or other parts of the GI tract are unlike the conditions found in the laboratory where gene transfer can readily be observed.

Potential harm

153. Wild native bird numbers could decline due to the exposure to infectious GM *E. coli* resulting in colibacillosis and death.

154. Harm to wild birds as a result of reversion to virulence and the subsequent infection of wild birds with this *E. coli* strain is unlikely. Colibacillosis caused by APEC is considered a secondary infection and mainly affects sick, stressed or otherwise weakened birds. Most poultry already carry *E. coli* serotype O1, O2 and O78 as part of their native gut flora. It is likely that wild birds have similar serotypes in their native gut flora. Recovery of the *aroA* gene will reinstate a wildtype form of the *E. coli* O78 strain. This strain is already present in the environment thus the potential harm of birds being exposed to GMO with recovered *aroA* gene is not greater than exposure of birds to the wildtype organism.

Conclusion

155. Risk scenario 2 is considered to be a negligible risk due to the causal pathway and the potential harm being highly unlikely to eventuate. In case of a reversion to virulence, the risks associated with the revertant virulent strain are unlikely to be greater than the potential risks associated with *E. coli* serotype O78 which is already present in the environment. Therefore, this risk is not considered to be a substantive risk that warrants further detailed assessment.

2.4.3 Risk scenario 3

Risk source	Causal pathway	Potential harm
GM *E. coli* O78 *aroA*-	Exposure of feral or pest birds to the GMO ⬇ Immunization of feral or pest birds ⬇ Increased survival and persistence of feral or pest birds	Increased number of feral or pest birds

Risk source

156. The source of the potential harm for this risk scenario is the GM *E. coli* O78 *aroA*-.

Causal pathway

157. During storage, transport and disposal birds could be exposed to the GM vaccine.

158. Feral or pest birds in contact with the GM vaccine may result in birds with immunity to *E. coli* O78. If environmental conditions were adverse for birds in general, then vaccinated birds may have an advantage over non-vaccinated birds.

Potential harm

159. Inadvertent vaccination of wild birds could lead to an increase in the overall number of pest birds.

Exposure of feral or pest birds to the GMO

160. There are several potential pathways for how pest species could come into contact with the vaccine. In the event of a spill during storage or transport birds might be able to swallow or inhale some of the lyophilised powder. Additionally, they could come into contact with the vaccine when unused vaccine is disposed of at the farm, during disposal of waste from the farm or when ingesting faecal matter from vaccinated birds.

Immunization of pest birds

161. For a successful challenge of pest birds with the vaccine, the titre of the GMO has to be high. Low levels of the GMO will not trigger an immune response. During safety testing the GM vaccine is routinely administered at a concentration of around 1×10^9 CFU/bird by oral gavage. The most likely scenarios where pest birds could get vaccinated are during disposal of unused vaccine, spillage of the lyophilised powder or by ingesting faecal matter from birds that have been recently vaccinated.

162. Spillage of lyophilised powder and pest birds ingesting the powder is unlikely as the spill can be controlled in a way that no pest birds will have access to the GM vaccine or the GMO will be diluted to the point where it will not trigger an immune response.

163. Vaccination of chickens with the GM vaccine will be performed according to the manufacturer's instructions. Zoetis would require an approval from the APVMA to supply the GM vaccine and if approval is granted, the vaccine is likely to be classified as a prescription animal remedy and would require supervision by a registered veterinarian to be used in commercial poultry farms. It will be administered indoors (this includes free range farms) and the birds must not be allowed access to any other water supply until vaccination is completed (ie all water is consumed or spraying is completed). The likelihood of pest birds having access to water containing the GM vaccine is highly unlikely.

164. The most likely way of pest birds getting access to the vaccine is by ingesting faecal matter from birds that were vaccinated recently. This is only the case for farms where free range chickens are raised/kept/bred or if pest birds get access to the untreated waste of commercial poultry farms. However, current poultry farming practices make it unlikely that pest birds will get access to untreated waste of vaccinated chickens. Therefore, the most likely way of pest birds being exposed to the GMO is by ingesting faecal matter from birds that were vaccinated recently.

165. Although immunisation of feral or pest birds may increase their survival and reproductive success, this would be limited to the vaccinated individuals.

Increased numbers of pest birds

166. By ingesting faeces from vaccinated birds and the resulting immunisation, pest birds could increase in numbers in the environment. Colibacillosis is considered a secondary infection in birds that are stressed or have been exposed to a primary infection. Compared to unvaccinated desirable species, vaccinated pest birds could survive in stressful circumstances or when being exposed to a primary infection. Additionally, pest birds could spread the GMO in the environment.

167. However, it is likely that the *E. coli* serotype O78 is already present in the environment and the GMO will not persist in the environment due to its replication deficiency.

168. For this scenario to lead to harm to the environment, a large number of pest birds would have to be vaccinated simultaneously. If colibacillosis was one of the main factors limiting the pest bird population then vaccination could get them some ecological advantage which could result in adverse effects on desirable species.

Conclusion

169. Risk scenario 3 is considered to be a negligible risk due to the causal pathway and the potential harm being highly unlikely to eventuate. Based on the available data, accidental exposure of the GM vaccine to a limited number of pest birds is not likely to have an effect on the environment. Therefore, this risk is not considered to be a substantive risk that warrants further detailed assessment.

Section 3 Uncertainty

170. Uncertainty is an intrinsic part of risk analysis[1]. There can be uncertainty about identifying the risk source, the causal linkage to harm, the type and degree of harm, the chance of harm occurring or the level of risk. In relation to risk management, there can be uncertainty about the effectiveness, efficiency and practicality of controls.

171. Risk analysis can be considered as part of a first tier uncertainty analysis, namely a structured, transparent process to analyse and address uncertainty when identifying, characterising and evaluating risk. However, there is always some residual uncertainty that remains. If the residual uncertainty is important and critical to decision making, then this residual uncertainty may be subjected to further analysis (second tier uncertainty analysis), such as building 'worst case' scenarios, or by using meta-analysis where results from several studies are combined.

172. There are several types of uncertainty in risk analysis (Bammer & Smithson 2008; Clark & Brinkley 2001; Hayes 2004). 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

[1] 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.

- perception – processing and interpreting risk is shaped by our mental processes and social/cultural circumstances, which vary between individuals and over time.

173. Uncertainty can also arise from a lack of experience with the GMO itself. In regard to the GM vaccine, the overall level of uncertainty is low given the several years of using the vaccine in United States and many other countries around the world. None of these uses have resulted in a confirmed incident for human health, safety or the environment. However, the GMO contains virulence associated genes, which may or may not be present in Australia. Therefore, for the current application there is uncertainty with respect to the following:

- Lack of Australian experience with commercial application of Poulvac *E. coli*® vaccine. It was first used on a commercial scale as a chicken vaccine in the United States (in 2006) followed by other countries including the recent EU (2012) release and to date there have not been confirmed reports of adverse effects caused by these authorised releases. A pharmacovigilence report produced by Pfizer Inc looked at the number of doses sold and the number of adverse event cases reported in the USA over a 4 year period. No confirmed cases of adverse reactions in humans or the environment were found.
- The pharmacovigilence report is based on data from the use of the vaccine in the USA. The receiving environment could be different in Australia. Therefore, there is some uncertainty associated with commercial release into the Australian environment. However, based on the available information relating to non-target effects on vertebrates, phenotypic characteristics and potential for an increased level of harm to people and the environment, no differences have been identified that would lead to increased estimate of risk associated with the release.
- There is a potential for changes to the genome and metabolites as a by-product of the 100 bp deletion of the *aroA* gene. *E. coli* O78 *aroA*- has not been fully sequenced and it is therefore not possible to say if there were any changes in the genome other than the intended ones. No analysis of metabolites of the GMO has been performed; it is therefore not possible to assess the effects of the *aroA* deletion on other metabolites directly. However, there has been a history of safe use of the GM chicken vaccine for both people, including those administering the vaccine to the chickens, and the environment.

Section 4 Risk evaluation

174. 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 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.

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

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

176. Three risk scenarios were postulated whereby the proposed dealings might give rise to harm to people or the environment. The level of risk for each of these scenarios was considered negligible in relation to both the seriousness and likelihood of harm and

considering both the short and long term. The principal reasons for these conclusions are summarised in table 1.

177. The nature and degree of uncertainty is not sufficient to affect the estimated level of risk for these scenarios.

178. 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. Therefore, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.

Chapter 3 Risk management

Section 1 Background

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

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

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

182. 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 of identified risks

183. The risk assessment of the risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed dealings with GM vaccine. The risk scenarios were considered in the context of the scale of the proposed dealings and the receiving environment. The risk evaluation concluded that no controls are required to treat these negligible risks.

Section 3 General risk management

184. 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
- identification of the persons or classes of persons covered by the licence reporting structures
- a requirement that the applicant allows access to specified sites for the purpose of monitoring or auditing.

3.1 Applicant suitability

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

- any relevant convictions of the applicant (both individuals and the body corporate)

- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

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

187. 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

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

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

189. Subject to approvals by other authorities, any person, including the licence holder, may conduct any permitted dealing with the GMOs.

3.4 Reporting requirements

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

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

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

192. 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

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

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

Section 4 Post release review

195. Regulation 10 requires the Regulator to consider the short and the long term when assessing risks. The Regulator does not fix durations, but 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.

196. For the current application for a DIR licence, the Regulator 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. This ongoing oversight will be achieved through post release review (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).

197. 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

198. 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), fax (02 6271 4202), 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 4.3 below) as well as the risk assessment of future applications involving similar GMO(s).

4.2 Requirement to monitor specific indicators of harm

199. 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.

200. 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. If specific indicators of harm were identified, the licence holder would be required to monitor these as mandated by the licence.

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

202. The characterisation of the risk scenarios discussed in Chapter 2 did not identify any risks that could be greater than negligible. Therefore, they did not warrant further detailed assessment. No specific indicators of harm have been identified in this RARMP for application DIR 125. However, specific indicators of harm may also be identified during later stages, eg through either of the other components of PRR.

203. 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

204. 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 needed managing, this could lead changes to the risk management plan and licence conditions.

Section 5 Conclusions of the RARMP

205. The risk assessment concludes that this proposed commercial release of GM vaccine poses negligible risks to the health and safety of people or the environment as a result of gene technology, and that these negligible risks do not require specific risk treatment measures.

206. General conditions have been imposed to ensure that there is ongoing oversight of the release.

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Appendix A Summary of advice from prescribed experts, agencies and authorities on matters relevant to the preparation of the consultation RARMP[2]

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

Summary of issues raised	Comment
If the GM vaccine were available in Tasmania would this be a breach of the GMO ban?	Information regarding the matter, including details of a state contact person and information on the review of the Tasmanian moratorium, was provided to the submitter via email.
Thanks for notification.	Noted.
Notes that the council policy regarding GMOs means that the area is GM free. No commercial poultry farms are in the area.	Noted.
Acknowledges receipt of notification and makes no further comments.	Noted.
Acknowledges receipt of notification and notes that no commercial poultry farms are in the area.	Noted.
States that the vaccine should not become an easy, costly and unsustainable solution to infection control caused through poor husbandry practices.	Commercial poultry farms follow best management practices. Zoetis would require an approval from the Australian Pesticide and Veterinary Medicines Authority (APVMA) to supply the GM vaccine. If approval is granted, the vaccine is likely to be classified as a prescription animal remedy and would require supervision by a registered veterinarian to be used in commercial poultry farms.
Wants the RARMP to examine if waste, including water, litter, faecal matter and carcasses, from vaccinated chicken will need special treatment or additional trade waste approvals.	Disposal of unused or waste material is discussed in Section 2.2.2 and risk scenario 3. The risk associated with waste was considered negligible Waste would be disposed of as per standard practice. Note that wastes (current and future) may contain chicken pathogens. Current farm practices are designed to ensure that pathogens are unlikely to re-infect chickens on-farm and that chicken and chicken products do not pose a danger to the health and safety of humans and safety of the environment. Composted waste might be used to produce fertilizer or similar products for commercial use. It is expected that low numbers – if any – of the E. coli survived in the litter and after composting.
Council is opposed to gene technology.	Noted.
Council is opposed to gene technology.	Noted.
Wants relevant precautions to ensure potential impacts from the GM vaccine to the environment are addressed.	Risk scenarios 2 and 3 address risks to the environment. These were assessed as negligible.

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

Summary of issues raised	Comment
Wants appropriate training, instruction and monitoring provided to farmers to ensure their thorough understanding of the storage, handling and disposal, as well as the risk involved with the use of the vaccine.	The Australian Pesticide and Veterinary Medicines Authority (APVMA) administers the Agricultural and Veterinary Chemicals Code Act 1994 to regulate agricultural and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade. Zoetis would require an approval from the APVMA to supply the GM vaccine. If approval is granted, the vaccine is likely to be classified as a prescription animal remedy and would require supervision by a registered veterinarian to be used in commercial poultry farms.
Proposes the RARMP reviews transfer of the GM vaccine from vaccinated poultry to other birds, particularly on free-range farms.	The potential harm from transfer from the GM vaccine to other birds is discussed in risk scenario 2 and 3. The risk of incidental infection of other birds was considered negligible.
The vaccine should not replace good management practices on chicken farms.	Commercial poultry farms follow best management practices. Zoetis would require an approval from the APVMA to supply the GM vaccine. If approval is granted, the vaccine is likely to be classified as a prescription animal remedy and would require supervision by a registered veterinarian to be used in commercial poultry farms.
Wants evidence for food safety upon consumption of vaccinated chickens.	Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment and food labelling, including GM food and residues. A study conducted in 2005 concluded that 'there has been no documented case of food-borne illness due to *E. coli* associated with consumption of poultry meat'. The Australian Pesticide and Veterinary Medicines Authority (APVMA) administers the Agricultural and Veterinary Chemicals Code Act 1994 to regulate agriculture and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade. As part of the assessment, the APVMA will consider the risk posed by the presence of residual vaccine in meat and eggs of chickens. As discussed in risk scenario 1, if the animals are exposed to the vaccine according to the manufacturer's instruction, the likelihood of GM *E. coli* being found in eggs is very low.
States that it is unclear which selection method was used in the final segregation step when creating the GM vaccine strain.	Noted. This information was provided by the applicant in appendix 1 of the application. In addition to approval by the Regulator, the applicant would also require a permit for import from the Department of Agriculture. The Department of Agriculture administers Australian biosecurity conditions for the importation of biological products under the Quarantine Act, 1908. These products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GM vaccines).
Wants to investigate if the DNA sequence that identifies an open reading frame on the complementary strand spanning the site of the deletion produces a novel product with biological activity.	No significant similarities to known genetic elements were found when performing a BLASTn search as discussed in Section 5.2 of Chapter 1. Five billion doses have been administered worldwide and there was no confirmed case of harm in humans and no reported adverse effects in animals.
Wants RARMP to assess if the genetic modification resulted in the production of toxic metabolites in the GM vaccine, eg from the ORF or through disruption of aromatic amino acid synthesis.	No metabolomics analysis of the GMO is available. As discussed in Section 2.1, the empirical evidence (5 billion administered doses and large scale field studies) suggests that there is no build-up of toxic substances in the GM vaccine.
Wants the RARMP to address if gain of function revertants may present a greater risk to the environment than the *E. coli* O78 currently present in Australia.	This is discussed in risk scenario 2 of chapter 2 of the RARMP. The risk associated with a reversion to virulence was considered no greater than that of the APEC wild type.

Summary of issues raised	Comment
Wants the RARMP to evaluate whether inadvertent vaccination of feral birds (as a result of shedding of the vaccine) could lead to localised environmental damage.	This is discussed in risk scenario 3 of chapter 2 of the RARMP. The risk associated with vaccinating feral birds was considered negligible.
Poultry systems can vary considerably between countries and, therefore, biosecurity issues may be more prominent in some production systems compared to others. Wants the RARMP to assess interaction with possible vectors such as wild birds and marsupials.	80% of the Australian poultry industry is under the control of two companies. They use a vertically integrated system with a high level of biosecurity. They need to protect the stock from predators and minimize the access of feral birds and other animals to feed and water. The most likely system where wild birds may get in contact with vaccinated chickens is on a free range chicken farm. This was discussed in risk scenario 3 of Chapter 2. The potential risk associated with wild birds being exposed to the GMO is discussed in risk scenario 2 of Chapter 2 of the RARMP and considered negligible. For more information about the Australian poultry industry please refer to Australian chicken meat federation (Australian chicken meat federation) and the Australian egg corporation limited (Australian egg corporation).
Wants possible interactions between this vaccine and other – prescribed – vaccinations evaluated.	The Australian Pesticide and Veterinary Medicines Authority (APVMA) administers the Agricultural and Veterinary Chemicals Code Act 1994 to regulate agriculture and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade.
Wants risks to human health and safety evaluated, particularly with view to staff administering the vaccine. Staff training and the use of personal protective equipment should be considered.	The exposure of humans to the vaccine was assessed in risk scenario 1 of chapter 2 of the RARMP. APECs are not considered to be human pathogens. Administration of the vaccine will be subject to regulation by the APVMA. Zoetis would require an approval from the APVMA to supply the GM vaccine. If approval is granted, the vaccine is likely to be classified as a prescription animal remedy and would require supervision by a registered veterinarian to be used in commercial poultry farms.
Wants consideration of contextual changes over time where poultry production systems will not be spatially isolated from urban areas and, therefore, exposure of people via aerosols and shedding may increase over time.	The risk to humans from the GM vaccine was assessed in risk scenario 1 of chapter 2 of the RARMP. APECs are not considered to be human pathogens. Indoor administration of the vaccine as well as common farm practices such as composting of chicken litter and removal of dead birds further minimise the potential of exposure to people via aerosol and shedding. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade.
Wants consideration of inadvertent revaccination of older chickens in poultry production with mixed age chickens.	The Australian Pesticide and Veterinary Medicines Authority (APVMA) administers the Agricultural and Veterinary Chemicals Code Act 1994 to regulate agriculture and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade. Revaccination could potentially only effect egg laying chicken (layers) as poultry raised for meat (broilers) would be vaccinated once and are generally not kept in mixed aged facilities. Layers could be revaccinated between 12 and 14 weeks of age, well before they reach maturity and start laying eggs (20 weeks).
Wants consideration of the likelihood of residual vaccine in chicken produce such as meat and eggs.	Food Standards Australia New Zealand (FSANZ) is responsible for human food safety assessment and food labelling, including GM food and residues. A study conducted in 2005 concluded that 'there has been no documented case of food-borne illness due to E. coli associated with consumption of poultry meat'. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade. As part of the assessment, the APVMA will consider the risk posed by the presence of residual vaccine in meat and eggs of chickens. As discussed in risk scenario 1, if the animals are exposed to the vaccine according to the manufacturer's instruction, the likelihood of GMO being found in eggs is very low.

Summary of issues raised	Comment
Doubts that there is sufficient evidence relating to reversion to pathogenicity in the application and wishes the RARMP to address this issue.	In case of a reversion to virulence the potential harm caused by the revertant is likely to be no more than the potential harm caused by the parent organism E. coli O78. This has been addressed in risk scenario 3 of chapter 2 in the RARMP.
Wants the RARMP to evaluate if there is evidence for genomic re-assortment and recombination in the GMO.	Laboratory studies, field trials and a history of safe use indicate that there has either been no genomic re-assortment or, if a genomic re-assortment has occurred, it has no deleterious effects on the GMO and has not resulted in harm to people and the environment. Potential harm through recombination was discussed in chapter 2, risk scenario 3 and considered negligible.
Has taken note of the submission but are not required to take any further action at the moment	Noted.
Provides information on the presence of O-serotypes of *E. coli* in Australia for the risk context.	Noted.
Wants the RARMP to consider the prevalence of Avian Pathogenic *E. coli* serotypes, and in particular type O78, in Australia.	*E. coli* serotype O78 was isolated in Australia in a 1987 study. Please refer to chapter 1, section 4 of the RARMP for more details.
Wants the RARMP to consider the properties of the parent organism with the potential to cause harm.	The parent organism is an avian pathogenic *E. coli* that was isolated from chicken that had died of colibacillosis. Please refer to chapter 1, section 5 of the RARMP for more details
Wants the RARMP to consider whether the genetic modification may alter levels of *E. coli* metabolites in a manner which may cause harm.	As discussed in Section 2.1 of Chapter 2, no metabolomics analysis of the GMO is available. The empirical evidence (5 billion administered doses and large scale field studies) shows no adverse effects and suggests that there is not build-up of toxic substances in the GM vaccine.
Wants the RARMP to consider the potential for the GMO to replicate and persist in the environment.	The GMO has limited potential to replicate and persist in the environment due to the genetic modification. Please refer to chapter 1, section 5 of the RARMP for more details
Wants the RARMP to consider the pathways and levels of exposure of wild birds to the GMO as a result of vaccination in poultry houses and free range farms.	Risk scenarios 2 and 3 of Chapter 2 of the RARMP address the potential for harm from exposure of wild birds to the vaccine which was considered negligible.
Wants the RARMP to consider the training requirements for persons administering the vaccine.	The Australian Pesticide and Veterinary Medicines Authority (APVMA) administers the Agricultural and Veterinary Chemicals Code Act 1994 to regulate agriculture and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are suitably formulated, are of acceptable quality, are properly labelled and, when used according to the instructions, are safe, efficacious and do not unduly prejudice trade. Zoetis would require an approval from the APVMA to supply the GM vaccine. If approval is granted, the vaccine is likely to be classified as a prescription animal remedy and would require supervision by a registered veterinarian to be used in commercial poultry farms.
Wants the RARMP to consider the results of GMO-specific post-marketing monitoring in the USA.	The results of pharmacovigilence study in the USA have been discussed in Section 5.2 of Chapter 1. No confirmed harm to humans and environment was reported.

Appendix B Summary of advice from prescribed experts, agencies and authorities on the consultation RARMP[3]

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

Sub. No:	*Summary of advice*	Comment
1	The council is concerned about the potential of *E. coli* (including uropathogenic (UPEC) and Shiga toxin producing enterohemorrhagic (EHEC) strains) to infect humans followed by development of severe disease symptoms in infected individuals such as haemolytic uremic syndrome (HUS) and diarrhoea.	The Act requires the Regulator to protect human health and safety and the environment by identifying and managing risks posed by or as a result of gene technology. *E. coli* O78 is a member of the avian pathogenic *E. coli* (APEC) pathogroup. APEC are not considered human pathogens and are not known to produce any toxins that affect humans. They are not the cause of HUS and it has not been shown that they cause diarrhoea in humans. GM *E. coli* O 78 is an attenuated from of the wild type strain that is replication deficient. Therefore, it has limited potential for spread and persistence in the environment and there has been no single confirmed case of harm to humans or the environment as a result of import, storage, transport or disposal (which are the dealings approved by this licence) when used overseas.
	If GM *E. coli* is introduced into chickens and the animal uses the majority of its antibodies to combat a disease pathogen it leaves itself vulnerable to any of its internal pathogens to attack the unprotected regions.	Small scale laboratory studies, large scale field studies and a history of safe use did not show any detrimental effects of the GM vaccine on chickens.
	The council strongly opposes the introduction of any genetically modified products into the food chain. The introduction of a modified product containing *E. coli* as a vaccine must be avoided as it has the potential to spread to the human consumer of the chicken.	The APVMA regulates agricultural and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are, when used according to the instructions, safe and efficacious. As part of the assessment, the APVMA will consider the risk posed by the presence of residual vaccine in meat and eggs of chickens. Zoetis would require an approval from the APVMA to supply the GM vaccine.
2	The council notes that from the information provided it would appear the regulatory controls in place are adequate. However, the council expects the WA Department of Health will also be consulted about this matter as the council relies on advice from the department relating to toxicology and the like.	The Western Australia Department of Agriculture and Food has been consulted with about the release of GM *E. coli* chicken vaccine. The Department in turn consults with other WA government departments and submits the advice received by these departments to the OGTR.
3	The council notes the RARMP and confirms its reasonable expectation that responsible State and Federal Agencies will provide or require suitable monitoring to ensure there are	Noted. The Act (and licence) requires that the licence holder reports any adverse effects to the Regulator in regards to the approved dealings (import, transport, storage and disposal).

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

Sub. No:	*Summary of advice*	Comment
	no deleterious effects to people or the environment from the commercial release of the GM vaccine (if approved). These agencies shall provide any necessary resources to respond appropriately in the event that any adverse impact is identified in the future.	
4	The RARMP seems to cover the concerns and the assessment seems to indicate there is no serious risk to public health, food safety or environmental risks. Notes that other agencies deal with food labelling.	Noted.
5	Do not have access to the specialist scientific information or advice to provide extensive comment. Licence conditions need to ensure that ongoing monitoring of the release is maintained.	Noted. Licence conditions require ongoing monitoring of the sites until they are signed off.
6	Acknowledges that the council is unable to provide qualified advice on this matter as the Council has no specialised skill set to assess the proposal. The council trusts that the proposal has considered all relevant health and environmental issues.	Noted.
7	Noted that the risk scenarios presented appear to pose negligible effect to both environmental and human health. The council does not have any policy in place opposing gene technology. Council will refer to FSANZ and the NSW food authority for guidance on the labelling of products containing GM products.	Noted.
8	Agrees with the overall conclusions of the RARMP; that the proposed GMO dealings pose negligible risk to the health and safety of people and the environment.	Noted
	Clarify and/or include further consideration of the current management practices for use of chicken manure as fertiliser, in the context of potential persistence and dispersal of the vaccine strain.	Additional information has been added to risk scenario 1 in chapter 2 of the RARMP. Current practices with chicken manure ensure that the consumers do not come into contact with human pathogens commonly found in chickens including *Salmonella* and *Campylobacter*.
	Issues regarding the potential exposure to people or other organisms other than vaccinated chickens should be raised with the APVMA	The OGTR and the APVMA are obliged to seek each other's advice on all applications for intentional release of a GMO into the environment. Issues regarding exposure to people or other organisms other than vaccinated chickens were discussed with the APVMA in the course of the preparation of this RARMP
	Clarify the characterisation of *E. coli* O78 as "not a human pathogen" in the context of current scientific evidence and understanding of the zoonotic potential of APEC strains, including the potential for subclinical infection and/or cause clinical disease in mammals, and inclusion of any additional relevant published evidence. Clarify the wording and	The wording in the RARMP has been amended to better reflect our current understanding of APEC. The RARMP is referring more specifically to the APEC strain O78. The zoonotic potential of *E. coli* O78 used to generate the GM vaccine or the GM *E. coli* has not been demonstrated. Infections of wild birds has been discussed in Risk scenarios 2 and 3 in chapter 2; no substantive risk could be identified.

Sub. No:	*Summary of advice*	Comment
	argumentation regarding potential for the *E. coli* O78 vaccine strain to result in zoonotic infections or infection of wild birds.	
	Consider whether there is any additional information regarding the potential for the vaccine strain to grow or persist in eggs, including in the context of the timing of vaccination and chicken production timeline.	This has been addressed in chapter 1 and chapter 2 of the RARMP. Current practices in the poultry industry ensure that the consumers do not come into contact with human pathogens commonly found in chickens including *Salmonella* and *Campylobacter*.
9	Is satisfied with the conclusion of the draft RARMP.	Noted.
10	Is supportive of this application as the consultation RARMP indicates that the proposed commercial release poses negligible risks to human health and safety of the environment. Notes that there are general licence conditions to ensure ongoing oversight of the release and that the vaccine is also subject to APVMA approval.	Noted.
11	Based on the RARMP, it is reasonable to conclude that there is no substantive risk for people or the environment associated with the vaccine.	Noted.
	Notes that the suggestion that APEC are zoonotic agents is based on shared virulence factors rather than epidemiologic evidence of actual spread. As APEC are already present in Australia, this vaccine is more likely to reduce the potential risk of APEC for humans. While there is a possibility of reversion to virulence, any such reversion will generate an APEC that is already present in Australia.	Information about APEC are discussed in Chapter 1. The potential for reversion is discussed in Chapter 2 and no substantive risk was identified.
12	Has no objection to the granting of a licence for application DIR 125	Noted.
13	Supports the OGTR's conclusion that the proposed dealing posed negligible risk to human health and safety and the environment.	Noted.
14	Does not have any comments to make on this licence application at this time. Notes that the evaluation does not cover food safety and labelling and that APVMA approval is needed.	Noted.
15	Has no concerns regarding this application and concurs with the view of the OGTR that this application poses negligible risk to persons or the environment.	Noted.

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

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

Abbreviations:

Issues raised: **AW**: animal welfare; **Con**: Consultation; **E**: environment; **GTTAC**: Gene Technology Technical Advisory committee; **H**: human health; **L**: licence; **RC**: risk context; **U**: uncertainty, including knowledge gaps; **VU**: Vaccine use.

Other abbreviations: **APVMA**: Australian Pesticides and Veterinary Medicines Authority; **GM**: genetically modified; **GMO:** genetically modified organisms; **RARMP**: Risk Assessment and Risk Management Plan.

Sub. No:	Issue	*Summary of issues raised*	Comment
1	-	Is strongly opposed to a GM vaccine for chickens.	Noted.
2	VU	Thinks that dealings with the GMO should not be approved because it is better for human health and the environment and animal welfare to have poultry raised under healthy conditions where this vaccination is unnecessary. Therefore, the "negligible risk" using the GM vaccine should be avoided.	Australia's state and territory governments have the primary responsibility under their respective legislation for animal welfare. The Regulator has concluded that the import, transport and disposal of the GMO as well as possession (including storage), supply or use of the GMO for those purposes represents a negligible risk to people and the environment. The use of the GM vaccine on chickens is regulated by the APVMA. An approval from the APVMA is required prior to supplying the GMO vaccine for use.
3	H, E	States that a reduction in the spread and persistence of the GMO (due to attenuation) is not sufficient to ensure the safety to human and animal health, and the environment. The GMO may survive in a variety of environments including wastewater, groundwater and chicken litter sold as garden manure.	In addition to several efficacy and toxicology studies, a pharmacovigilence report produced by Pfizer Inc investigated the number of doses sold and the number of adverse event cases reported in the US over a 4 year period. No confirmed cases of adverse reactions in humans or the environment were found. In an environmental persistence study GM E. coli vaccine could not be detected 42 days post vaccination. This has been covered in Chapter 1 and 2 of the RARMP. Based on the available data, the Regulator has concluded that dealings with the GMOs proposed for release represent negligible risks to people and the environment
	H, RC	Asks for a withholding period on the sale and use of chicken litter from vaccinated chickens.	The wording in risk scenario 1 was clarified to emphasise current biosecurity measures in the poultry industry.
	H	Claims that two toxicology studies are unacceptable as evidence of safety.	The conclusion of the risk assessment is not only based on these studies. The Regulator took into account a range of relevant information including, for example, the history of safe use of the GM vaccine, when concluding that the dealings pose negligible risk.
	H	States that the RARMP and licence application do not offer an evaluation or explanation of the	The Act requires the Regulator to consider risks to health and safety of people and the

Sub. No:	Issue	*Summary of issues raised*	Comment
		relative merits of a live vs killed vaccine.	environment that may be posed by dealings with the GMOs. Comparative merits of different technologies are outside of the scope of the Regulator's risk assessments.
	E	Asks if pecking could be a route of transmission as tissues of a bird infected with the GMO can transmit the organisms to another bird, travel to the organs of the recipient bird and be found there at least seven days later.	The mode of transmission of APEC is generally through the oral-faecal pathway or through the respiratory system. As discussed in chapter 1, *E. coli* in chickens is usually found in the upper and lower intestines of the birds, the likelihood of oral transmission via pecking is low.
	H	Wants data on the incidence of GMOs on eggshells in commercial environments before authorising application of the vaccine. States that visual inspection in a high-volume commercial operation offers inadequate assurance of nil transmission of the GMO and asks to mandate evidence on the incidence of this GMO and other bacteria in a large sample of eggs. States that a withholding period [for chicken products] needs to be mandated rather than recommended and asks if mass vaccinations would be permitted.	The APVMA regulates agricultural and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are, when used according to the instructions, safe and efficacious. As part of the assessment, the APVMA will consider the risk posed by the presence of residual vaccine in meat and eggs of chickens. Zoetis would require an approval from the APVMA to supply the GM vaccine.
	E	States that the RARMP and licence application fail to substantiate any need for the GM poultry vaccine.	The Act requires the Regulator to consider risks to the health and safety of people and the environment posed by the dealings with GMOs. Whether there is a need for the GMO or not is outside of the scope of the Regulators assessments.
	RC	States that the RARMP provides no assessment concerning the incidence of *E. coli* O78 in chicken flocks managed under various conditions.	The RARMP includes relevant information as part of the risk context.
	AW	Raises concerns about the standard of poultry keeping, including: • The use of the GM vaccine may favour poor quality poultry feeding and housing • The use of routine, non-veterinary use of antibiotics in chicken husbandry should be prohibited • Poultry farms do not always follow best management practices.	Australia's state and territory governments have the primary responsibility under their respective legislation for animal welfare. The Regulator has concluded that the permitted dealings are import, transport and disposal of the GMO as well as possession (including storage), supply or use of the GMO for these purposes of those dealings represents a negligible risk to people and the environment. The use of the GM vaccine on chickens is regulated by the APVMA. An approval from the APVMA is required prior to supplying the GMO vaccine for use.
	VU	Claims that the RARMP implies that the vaccine will be used for selective treatment of disease. Elsewhere, the RARMP claims that the vaccine will be routinely administered to all birds in a commercial environment.	Vaccines are routinely used to prevent diseases. Zoetis would require an approval from the APVMA to supply the GM vaccine as well as approval from other regulators.
	Con	Claims that Appendix A in the RARMP is dismissive of questions and objections from prescribed experts, agencies and authorities.	Each comment was considered and those within the scope of the Act were addressed in the RARMP (as indicated in the responses).
	GTTAC	Wants advice from the Gene Technology Technical Advisory Committee (GTTAC) to be made publically available.	A communique from each GTTAC meeting is made publicly available. In addition, advice from all prescribed agencies and GTTAC is included in Appendices A and B of the final RARMP.

Sub. No:	Issue	*Summary of issues raised*	Comment
	GTTAC	Criticises that no ecologist is a member of GTTAC.	Members of GTTAC have a broad range of skills and expertise relevant to this application. In addition, the Regulator has consulted widely on the draft RARMP, including the Environment Minister, other regulatory agencies, State and Territory agencies, and the public.
	L	Questions the general reporting requirements proposed in the licence and proposes to require the applicant to report on • the presence of the GMO on the surface or interior of eggs after vaccination • the health effects of the GMO in chickens, including reproduction, allergy and cancer • the exposure of people to the GMO via meat and eggs • the occupational exposures to the GMO from contact with faeces, litter, dust, eggs, carcasses, meat etc.	The APVMA regulates agricultural and veterinary chemical products, including vaccines. The APVMA ensures that vaccines for use in Australia are, when used according to the instructions, safe and efficacious. As part of the assessment, the APVMA will consider the risk posed by the presence of residual vaccine in meat and eggs of chickens. Zoetis would require an approval from the APVMA to supply the GM vaccine.
	U, H, E	Agrees that the RARMP identifies a number of knowledge gaps and other uncertainties, including • The genome of GM *E. coli* has not been sequenced • No analysis of metabolites was performed with the GMO and it is not possible to assess the effects of the *aroA* deletion on any other metabolites directly • The potential of replication of the GMO in eggs is limited but the level of limitation is unknown • Unintentional changes in the genome of the GMO may be present as a result of gene technology. • Questions why there was no study carried out in Australia to address uncertainty regarding effects on the Australian environment. • Wants a study regarding weight gain at up to 42 days post-vaccination.	Uncertainty is an intrinsic part of risk analysis - there is always some uncertainty. If the uncertainty is important and critical to decision making, then it may be subjected to further analysis such as 'worst case' scenario building or by using meta-data analysis. For the licence application several uncertainties were identified and clearly outlined in the RARMP. After careful analysis of all the information available no risk associated with these uncertainties could be identified that would be greater than negligible to the health and safety of people and the environment. This has been addressed in Chapter 2, section 3.