This Memo has been cleared by Frank Clinton for submission to the Board
Signed: Dated:
Bea Claydon 4 Apr 2013

Environmental Protection Agency
An Ghníomhaireacht um Chaomhnú Comhshaoil

OFFICE OF CLIMATE,LICENSING & RESOURCE USE

INSPECTOR'S REPORT

TO:	BOARD OF DIRECTORS
FROM:	Bernie Murray - Environmental Licensing Programme
DATE:	4 April 2013
RE:	Notification from Merck Sharp Dohme acting on behalf of Intervet International B.V., Wim de Körverstraat 35, NL – 5831 AN Boxmeer, under Part II of the GMO (Deliberate Release) Regulations (S.I. 500 of 2003) to conduct a veterinary trial using a genetically modified vaccine (GMO Register No: G0493-01).

Applicant:	Merck Sharp & Dohme acting on behalf of Intervet International B.V., Wim de Körverstraat 35 NL – 5831 AN Boxmeer The Netherlands
GMO Register Entry No:	G0493-01
SNIF No[1]:	B/IE/12/02
Notification under Article 14(1) of S.I. No 500 of 2003:	The deliberate release of a genetically modified organism for purposes other than placing on the market (Part B Release – Veterinary Trial).
Timeframe for EPA's Decision under Article 18(5) of S.I. No 500 of 2003:	A person shall not deliberately release a genetically modified organism (GMO) for purposes other than placing on the market unless consent in writing has been granted by the EPA. The EPA shall communicate its decision (either grant consent with or without conditions or refuse consent) in writing to the notifier within 90 days of receipt of the notification.
Date of receipt of notification under Article 14 of S.I. No 500 of 2003:	14 December 2012
Request for additional information under Article 19 of S.I. 500 of 2003:	26 February 2013 14 March 2013
Additional Information submitted under Article 19 of S.I. 500 of 2003:	19 March 2013
Date by which decision is required:	12 April 2013

[1] The Summary Notification Information Format (SNIF) is a summary of the notification. It is forwarded to the European Commission for circulation to all member states.

Representations to the EPA relating to this notification under Article 16 of S.I. 500 of 2003:	30

Introduction

Merck Sharp Dohme (MSD) acting on behalf of Intervet International B.V., Wim de Körverstraat 35, NL – 5831 AN Boxmeer, sought the consent of the Environmental Protection Agency (Agency) on 14 December 2012 to conduct a veterinary trial in foals. The trial will involve the administration of a vaccine ('Equilis RhodE') containing genetically modified (GM) bacterium, *Rhodococcus equi*, to foals in order to establish if it is effective in preventing infection by *R. equi*.

The notifier proposes to conduct this trial at Belmont Stud Farm, Belmont, Co Offaly. The notification was made in accordance with Part II of the GMO (Deliberate Release) Regulations (S.I. 500 of 2003)[2].

Rhodococcus equi (*R. equi*) is a gram positive bacterium that lives in a broad range of environments including soil, water and eukaryotic cells.

It is the causative agent of a severe respiratory disease of horses that is a leading cause of mortality in foals. Rhodococcal pneumonia typically affects foals between 1 and 6 months of age. The foal becomes infected through inhalation of contaminated soil dust while grazing or from the breath of infected animals. The organism invades, survives and multiplies within the alveolar macrophages in the lung which leads to the development of abscesses in the lung and lung tissue destruction.

R.equi can also multiply in the intestine contributing to its dissemination via an oral-faecal cycle that enriches the farm environment with virulent strains. The organsim has a long survival period in manure and soil, typically 6 months. Rhodococcal pneumonia in foals is generally fatal if antibiotic treatment is not rapidly administered.

The organism becomes endemic on stud farms where it presents a challenge since *R. equi* is resistant to many antibiotics (e.g. penicillins, cephalosporins, sulfamides, quinolones, tetracyclines, clindamycin and chloramphenicol). At present there is no effective vaccine available.

R. equi can also infect non-equine species. *i.e.* humans, pigs, cattle, sheep, goats, cats, and dogs. However, disease caused by *R.equi* infection in foals is by far the most devastating and it is recognised as one of the most important infectious problems that can afflict the equine industry worldwide.

R. equi is also an opportunistic human pathogen associated with AIDS and immunosuppression. Human Rhodococcal infection is clinically and pathologically similar to pulmonary tuberculosis.

[2] The Agency decided in October 2001 to regulate veterinary/clinical trials under the deliberate release legislation.

This is the first veterinary trial to be carried out under this legislation in Ireland. The Agency previously issued consent to Schering Healthcare Ltd (G0134-01) in 2002, Applied Genetic Technologies Corporation (G0362-01) in 2010, and Professor Samuel McConkey, Department of Tropical Medicine and International Health, RCSI, Dublin (G0451-01) in 2011, to perform clinical trials on human medicinal products.

A 2012 report on the importance of the Irish Sport Horse sector concluded that the sport horse population in Ireland was estimated to be 124,000 equines contributing €708 million per annum to the Irish economy. Over 47,000 people are estimated to be involved in the industry.

Considerations for the Board

Role of the EPA in reviewing this notification

The Agency's responsibility under the Genetically Modified Organisms (Deliberate Release) Regulations (Article 18, S.I. 500 of 2003) is to evaluate the risks posed by the proposed deliberate release for human health and the environment and to give consent to the release only if satisfied that it will not result in adverse effects on human health or the environment.

Description of the Genetically Modified Micro-Organisms for use in the proposed clinical trial

The GM strain of *Rhodococcus equi* contained in the vaccine 'Equilis RhodE' is *R. equi* strain RG2837 (hereafter referred to as the vaccine strain). The vaccine strain derived from parent strain *R. equi* RE1 is a deletion mutant. These deletions prevent cholesterol metabolism as a result of which the vaccine strain is unable to grow/survive within macrophages in the lung and is thereby no longer able to cause the illness. The vaccine strain does not contain foreign DNA aside from 12 nucleotides left over from the vector used to construct the vaccine strain.

Purpose of the proposed deliberate release

The objective of the veterinary trial is to assess the efficacy of the vaccine 'Equilis RhodE' in foals under field conditions.

Proposed location of the deliberate releases

The proposed location for the deliberate release is Belmont Stud Farm, Belmont, Co Offaly.

Timeframe for the proposed clinical trial

The notification requests performance of the veterinary trial at the above named location from 13 March 2013 to 30 September 2016 (i.e. 4 foaling seasons 2013, 2014, 2015 and 2016).

The notifier conducted a risk assessment in accordance with Article 5(2) and the Second Schedule of the GMO (Deliberate Release) Regulations, S.I. No 500 of 2003.

The wild type *R. equi* strain

The wild type strain of *Rhodococcus equi* is classified as a group 2 biological agent[3] under Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work.

Foals are infected by *R. equi* while grazing or by the inhalation of soil dust contaminated with *R. equi* which is believed to be the primary route of exposure. The infection can lead to the development of extensive bronchopneumonia. Most bacteria remain in the lung abscesses, but low level shedding does occur by coughing, sneezing and/or exhalation.

Approximately half of all foals infected with Rhodococcal pneumonia also develop ulcerative colitis which may manifest clinically as diarrhoea. Intestinal disease rarely occurs in the absence of pneumonia and probably results from the ingestion of large amounts of contaminated sputum.

Foals shed large numbers of virulent *R. equi* in their manure but the numbers decline after 6 weeks of age. Ingestion of *R. equi* (as distinct from inhalation) does not usually result in disease, but in immunization. As a result of this process, older foals and adult animals have antibodies against *R. equi* and rarely get infected.

R. equi is considered a pathogen for immunocompromised people, especially those with acquired immunodeficiency syndrome (AIDS) where it acts as an opportunistic pathogen most commonly manifesting as necrotising pneumonia. According to the notifier *R. equi* rarely infects immunocompetent humans.

Pathogenicity in the wild type R. equi strain

The capacity of the wild type strain of *R. equi* to survive and multiply in macrophages in the lung is central to its pathogenicity.

Wild type pathogenic strains of *R. equi* can carry large virulence plasmids (Vap) which are essential for reproduction in macrophages. These virulence plasmids are species specific with $VapA^+$, $VapB^+$ and $VapAB^-$ being associated with horses, pigs and cattle respectively. It is not possible for different virulence plasmids (e.g. $VapA^+$ and $VapB^+$) to coexist in the same strain of *R. equi*, they are mutually exclusive. In contrast, all three plasmid types, as well as strains with no virulence plasmids may be found in *R. equi* strains isolated from humans suggesting that *R. equi* is zoonotic[4].

[3] "group 2 biological agent means one that can cause human disease and might be a hazard to workers; it is unlikely to spread to the community ; there is usually effective prophylaxis or treatment available" – Directive 2000/54/EC.

[4] 'Zoonosis' means any disease and/or infection which is naturally transmissible directly or indirectly between animals and humans.

Transfer of virulence plasmids between *R. equi* strains can occur naturally through a process called conjugation.

The vaccine strain of *R. equi* will carry the VapA$^+$ virulence plasmid rendering it equine specific. The virulence plasmid is not genetically modified. The VapA$^+$ virulence plasmid may be transmitted to plasmid free (non-pathogenic) *R. equi* strains rendering them pathogenic. However, this would present no greater risk than exposure to naturally occurring pathogenic strains. Furthermore, the transfer of virulence plasmids would not entail the transfer of new or foreign genetic material.

The recombinant vaccine strain

The parent strain is *R. equi* strain RE1[5]. Four genes at two loci, *ipdAB1* and *ipdAB2*, were deleted from the chromosome of *R. equi* RE1 to yield the vaccine strain *R. equi* RG2837 using recombinant DNA techniques. The vaccine strain is therefore a deletion mutant and contains no foreign genetic material with the exception of 12 nucleotides left over from the vector used to construct the vaccine strain.

The deleted genes are involved in cholesterol metabolism and appear to have a role to play in the survival of *R. equi* in macrophages in the lung. Removal of these genes renders the vaccine strain unable to grow within macrophages consequently it will not cause pneumonia in horses but it can impart immunity against *R. equi*. The vaccine strain has thereby lost its capacity for pathogenicity and is strongly attenuated in the host compared to the wild type strain.

Gene Transfer

Reversion to the virulent wild type phenotype or the creation of a more virulent strain through the exchange of genetic material is deemed unlikely because the genetic modification involves a chromosomal deletion rather than from the mobile element i.e. the plasmid.

Risks to the Environment

The wild type *R. equi* strain is already ubiquitous in the environment[6]. Although it varies from foal to foal, the vaccine strain of *R. equi* can be excreted intermittently into the environment in the manure of vaccinated foals for up to 4 weeks following vaccination. The vaccine strain does not contain foreign DNA. Therefore in the event that the vaccine strain is excreted into the environment no new genetic elements will be introduced into the environment.

No difference in survivability is expected between the vaccine strain and the wild type strain (as demonstrated by spiking experiments) rather the vaccine strain is expected to behave similarly to the wild type strain.

Belmont Stud Farm, Co Offaly is the location for this proposed trial. It is situated in an agricultural area close to Belmont village in Co Offaly. Vaccination will take

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[5] The parent organism is a facultative pathogenic soil saprophyte i.e. it possesses a mechanism for damaging and infecting susceptible host tissue by growing in the gut. It lives on decaying or dead organic matter in the environment

[6] *R. equi* has been shown to be present to a level of 4×10^3 to 1×10^4 cfu/g soil in the vicinity of horse farms.

place within the 'release site' on the stud farm. The release site comprises the foal shed (where vaccine administration will take place), the stables, the yard, the Pony Garden, The Orchard and the Roadfield, an area approximately 1.11ha in size. The foals (and mares) will be held within the release site for approximately 28 days. Peak shedding occurs over a period of 1 – 2 days following each vaccination. A period of reduced shedding follows, the duration of which can vary from foal to foal. As long as shedding continues within the release site, faeces and litter (bedding) will be collected and removed for decontamination by SRCL Ltd. The foals will not be moved from the release site into the paddocks (Big Field / Mill Field) until such time as a rectal swab is deemed 'clean' for the *R. equi* vaccine strain.

At a later stage in the trial, approximately 4 weeks after last vaccination, the foals will be moved to the 'Front Field' adjacent to a tributary of the River Shannon and according to the applicant the horses use it for drinking. The River Shannon is the closest supply source of water for Belmont Stud Farm through the Banagher Regional Water Supply. The nearest special area of conservation (SAC) is Clara Bog located 22km from the release site.

Wild type *R. equi* does not produce spores but it is capable of surviving for long periods in soil and manure in the environment typically up to 6 months. Optimal environmental conditions for growth of *R. equi* include neutral to moderate alkaline soil at 30°C enriched with horse manure. *R. equi* is inactive at temperatures below 10°C. Wild type *R. equi* is ubiquitously present in the environment and is endemic on this stud farm. The attenuated vaccine strain is expected to behave the same as the wild type strain in the environment and is not expected cause any greater environmental impact.

Cattle are maintained on farms adjacent to the release site. Badgers, foxes, rabbits and rodents occur in the surrounding countryside. The *R. equi* vaccine strain carries the VapA$^+$ virulence plasmid which renders the vaccine strain specific to horses. Given this specificity, it is expected that the *R. equi* vaccine strain would be unable to cause disease in other animal species. Studies were carried out by the notifier to demonstrate that the vaccine strain does not infect chickens, rats, mice, pigs or calves. Moreover, mares visiting the stud for natural breeding with a stallion will not be permitted during the vaccination programme.

Risks to Human Health

According to the applicant, immunocompetent humans are rarely affected by wild type *R. equi* while it can cause pneumonia in humans with compromised immune systems (e,g, AIDS patients, transplant patients). The development of *R. equi* as an opportunistic pathogen was accelerated by the spread of the AIDS pandemic.

While the virulence plasmids harboured by *R. equi* strains are mutually exclusive in animal species, all three plasmid types, as well as strains with no virulence plasmids may be found in *R. equi* strains isolated from humans. Therefore, it follows that the attenuated *R. equi* vaccine strain could cause infections in immunocompromised humans. However, the vaccine strain was shown to be less able to survive in human macrophages[7] it can be expected that the risk for

[7] Attenuation was confirmed in a human macrophage cell line

immunocompromised persons is at most equal but more likely less, compared to the wild type *R. equi*.

Although persons handling horses are very frequently exposed to this pathogen, *R. equi* infections in humans are rare and occur almost exclusively in immunocompromised individuals. *R. equi* infection was first described in humans in 1967. Only 19 cases of *R. equi* infection have been reported in immunocompetent humans in the period up to 2000[8]. Infection is primarily acquired through the respiratory tract but has been found to be acquired both by the oral route and by traumatic inoculation or superinfection of wounds. Epidemiological features, such as history of exposure to livestock or contamination with soil that may have led to *R. equi* infection were found in 10 of the 19 patients.

A number of measures have been implemented under Condition 6 to ensure that staff are informed of the health hazards associated with *R. equi* bacterium and in particular in relation to immunocompromised persons, to minimise the number of people coming into contact with the GM vaccine, the area in which it will be used and the vaccinated foals. Most notably, veterinary trial staff will be provided with a product brochure and will be informed both verbally and in writing of the potential health hazards associated with *R. equi* bacterium in general and in particular in relation to immunocompromised persons. The PI will seek to identify any immunocompromised staff members before commencing the trial. Access to the release site will be limited to veterinary trial staff and all visitors to the stud farm will be required to sign a visitors log.

Method(s) & quantities to be used for the release and subsequent shedding

Vaccination

The proposed trial will take place on one stud farm over the course of 4 foaling seasons (2013, 2014, 2015 and 2016).

Each season, in a blind study[9], 1 group of 25 foals will be vaccinated with the GM vaccine 'Equilis RhodE' (vaccinates) and a second group of 25 foals will remain unvaccinated (controls). Therefore, over the course of the trial it is envisaged that there will be 75 vaccinated foals and 75 untreated control foals. The foals will be vaccinated when they are 7 days old. In the first few days of life, the foals will be kept with the mare in individual stables. As soon as the weather permits, the mares and foals will be moved into the fields and a number of mares/foals will occupy the same field(s). The foals will not be weaned until they are 6 months old.

The GM vaccine will be administered to foals via the rectal route. The vaccinates will each receive a maximum of 4 doses of vaccine. Each vaccine dose will contain between 5×10^9 and 1×10^{11} cfu (colony forming units) of *R. equi* strain RG2837 (vaccine strain).

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[8] Clinical Infectious Diseases 2001: 32e39-47. *Rhodococcus equi* Infections in Immunocompetent Hosts: Case Report and Review. Kedlaya et al

[9] Blind study – in this instance the vaccine and a placebo will be taken in and administered to the foals as products A and B. Only the notifier (Intervet International B.V.) will have a list of foals in receipt of the vaccine.

Two doses of vaccine will be administered on two consecutive days when the foals are 7 days old and another two doses will be administered on two consecutive days 14 days later. Vaccination should be complete before the foal is 1 month old.

Vaccination will take place in the pony shed within the 'release site'.

The following **Table 1** shows vaccination, shedding (peak and reduced) and the movement of the foals between the release site and the paddocks over an 8 week period.

Week	1				2		3			4	5	6	7	8
Day	Day 1	Day 2	Day 3-4	Day 5-7	Day 8-13	Day 14	Day 15	Day 16 - 17	Day 18 - 21	Day 22 - 28				
Vaccination / Shedding	V1	V1	Peak Shedding	Reduced Shedding		V2	V2	Peak Shedding	Reduced Shedding					
Location	RS	RS	RS	RS	RS	RS	RS	RS	RS	RS	P1	P1	P1 & P2	P1 & P2

Vaccination (V): V1 is first vaccination; and, V2 is second vaccination.
Location:
Release Site (RS) corresponds to an area of 1.11ha and includes the foal shed, stables, the yard, the Pony Garden, the Orchard and the Roadfield , S14901063 on the aerial map (Appendix 1);
Paddocks (P):

- P1 = Big Field and Mill Field, corresponds to S14901062 on the aerial map (Appendix 1);
- P2 = corresponds to S14901074 on the aerial map (Appendix 1).

Post-vaccination and shedding

Vaccinated foals will be held within the 'release site' for at least 2 weeks after the last vaccination. Peak shedding is expected to occur on days 1 and 2 after each vaccination. Some foals will then stop shedding while others will show an low level of shedding up to several weeks after vaccination – it varies from foal to foal.

If, in any one year, 25 foals receive 4 vaccinations (as indicated) the maximum release level will be 1×10^{13} cfu. Theoretically the minimum number of bacteria shed will be equal to the inoculum but depending on multiplication in the animal and the period of time over which the foals are shedding, this value might be increased by 1, 2 or even 3 logs.

The point at which shedding ceases has not been determined and therefore it cannot be excluded that the vaccine strain could be shed at very low levels over a long period of time. In any case the vaccinated foals will not be moved from the release site until such time as a rectal swab is 'clean' for the *R. equi* vaccine strain.

Waste treatment

Each year, following the period of peak shedding (1 – 2 days after each vaccination) straw and litter (amounting to $1000m^3$ in total per foaling season) will be removed mechanically from the stable into closed containers and the release site (i.e. the stable and the concrete area in front of the stable) will be cleaned with a standard concentration of Steri-7 disinfectant. The straw and litter will be inactivated by SRCL Ltd (G0163-01).

In addition, some 300 empty vaccine vials and some 320 used syringes will be heat treated or alternatively treated by immersion in Steri-7 disinfectant, prepared in accordance with the manufacturer's instructions.

Storage & Preparation of the GMM prior to release/administration

The vaccine will be taken in from the Netherlands and presented as a freeze dried tablet in individual dose vials. It is reconstituted in 3ml of solvent prior to administration and the full amount of reconstituted vaccine is required for vaccination.

Worker protection measures taken during the release

There will be 6 – 12 people working on the stud farm during the course of the proposed trial of which the same 1 – 5 persons will come into contact with the trial foals.

Workers at the stud farm are already in frequent contact with the wild type *R. equi* strain which is endemic at the farm. Nonetheless, all veterinary trial staff will be issued with a product brochure which will explain the nature of a GMO and the safety measures that will be observed on the stud farm while the trial is ongoing. Condition 6 requires that all veterinary trial staff be informed both verbally and in writing of the potential hazards associated with *R. equi* bacterium in general as well as in relation to immunocompromised persons.

The vaccine will be administered rectally by a vet who will not be exposed to the vaccine strain. Since no needles are used, self-injection can be excluded.

Unauthorised persons will not be allowed to enter the release site while all visitors to the stud farm will be required to sign a visitor's log, (Condition 6).

Information about previous releases with these GMMs

The same GM vaccine was notified for release in the Netherlands (B/NL/09/004) and in Germany (B/DE/10/213).

The Dutch authorities gave a positive opinion and concluded that the risks for human health and the environment were negligible. Under the Dutch licence (PorM/RB IM 09-004) published on October 4th 2010 two studies were performed in 80 foals in the Netherlands in 2011 and 2012. No adverse effects relating to the vaccine were observed during these studies.

The German authorities also issued a license for deliberate release of the vaccine (BVL 107/2012/4). The study in Germany is scheduled to start in March 2013.

Duration and frequency of monitoring

Animal Health Monitoring

Both the vaccinated and control foals will be monitored from first vaccination until the last vaccinated foals reach the age of 6 months at which point the foals will be weaned.

The animals will be monitored daily for clinical signs of disease for 14 days after each vaccination (the animals will be monitored by the vet on days -1, 0, 1, 4, 7 and 14 and by stud farm staff on the intervening days). If clinical signs possibly related to *R. equi* infection are observed then the lungs will be scanned by ultrasound to determine the presence and size of abscesses. Clinical signs include general demeanour, feed intake, faecal consistency and rectal temperatures.

Thereafter the foals will be examined by ultrasound scan every two weeks for the presence of lung abscesses.

Following on from the trial, most of the trial animals will be sold either as a foal after weaning or later on in life.

Environmental Monitoring

The release site and the paddocks where vaccinated foals are present will be observed for signs of disease and other abnormalities. Abnormalities in the environment like mortality of wild animals will also be monitored for and recorded.

Dissemination of information and timeframe for making representations

Dissemination of information

The application was received by the EPA on 14 December 2012. The notice informing the public of submission of the application to the Agency was published in the 'Irish Horse' section of the Farmer's Journal dated 5th January 2013. Article 15 of the Regulations requires publication of this notice '*in a newspaper circulating in the area of the proposed deliberate release*'. Therefore publication in the Farmer's Journal meets this legislative obligation.

Article 15 also requires publication within 14 days of receipt of the application by the Agency. There was a 3 week period between receipt of the application on 14/12/2012 and publication of the notice on 05/01/2013. This was owing to section 87(13) of the EPA Act 1992 and amendments which states as follows:

'When calculating the appropriate period or any other time limit under this Act or in any regulations made under this Act the period between the 24th day of December and the 1st day of January, both days inclusive, shall be disregarded'.

Taking this section into consideration, the notice was published within 14 days of receipt of the application by the Agency and thereby meets with the requirements of article 15.

Making of Representations

In accordance with article 16 of the Regulations, members of the public are given 28 days within which to make representations to the Agency. Articles 16 and 48 of the Regulations require persons making representations to pay a fee of €10.

Shortly after publication of the notice, the applicant requested that certain of the information submitted be kept confidential. In accordance with Article 10, *'the Agency shall ... decide which information (if any) shall be treated as confidential information'*. Article 10(4) stipulates that certain information shall not be deemed confidential, notably: the name and address of the notifier; the location of the deliberate release; the purpose of the deliberate release; the description and intended uses of the GMO involved; methods and plans for monitoring the GMO and for emergency response; the ERA; or, any information or other matter referred to in article 22(1) (relating to the power of the Agency to modify, suspend or terminate consent).

The extent of confidential information was not finalised until 22nd January 2013 which in turn delayed the availability of non-confidential information to members of the public who had at the same time requested information on the proposed trial from the Agency. While awaiting the provision of non-confidential information, members of the public were furnished with the SNIF (Summary Notification Information Format) and the GMO Register Entry. The latest date for receipt of representations was 1st February 2013. This meant that the public had access to the full application (less confidential information) for 11 days as opposed to the required 28 days. At the end of the public participation period, the Agency had received 28 valid representations, a number of which highlighted the fact that the time given to the public to prepare and participate effectively was insufficient.

In an effort to rectify this situation and on foot of legal advice, the Agency wrote to all persons who had made a request for information or submitted a representation (valid or invalid) within the 28 day period commencing 5 January. These persons were provided with a renewed opportunity to consider the notification (less confidential information) and amend their existing representation or withdraw their existing representation and make a new representation. It provided persons who had made invalid representations or enquiries with an opportunity to submit valid representations and the Agency duly received 2 new representations. It was not however extended to persons who had not previously been in contact with the Agency in connection with this proposed trial.

Therefore, every effort was made in order to ensure that the public was not deprived in relation to participation in the process.

Representations made in respect of the notification

The Agency received 30 representations relating to this notification and proposed trial.

1. **Research relevant to GM vaccines is insufficient (as evidenced in notification under section 1.1.2.A.9 where notifier states "... very little work is done on the transfer of the R. equi plasmids"). The only**

research available at present originates from companies developing GM vaccines and is not independent. The effect on the environment is lethal and surely irreversible.

Agency's response

Section 1.1.2.A.9 deals with the host specificity of virulence plasmids. The vaccine strain *R. equi* RG2837 carries the VapA$^+$ virulence plasmid which renders the vaccine strain specific to horses. The virulence plasmid of the vaccine strain is identical to that of the parent strain. It has been demonstrated that it can be transferred by conjugation to a non-pathogenic recipient rendering the latter pathogenic. The virulence plasmid is not genetically modified. Given that the attenuation is not located on the virulence plasmid but on the chromosome, the mobilisation of this plasmid does not pose an increased risk.

This organism is considered GM because genetic engineering techniques were used to delete genes. No foreign genes were introduced into the organism. Therefore, the vaccine strain will pose no greater environmental risk that the wild type strain which is already ubiquitous in the environment.

2. What are the results of contained trials of this particular vaccine?

Agency's Response

Contained use trials were performed at the MSD Animal Health farm in St. Anthonis, The Netherlands. The GM vaccine did not cause any disease in the contained use studies and safety and efficacy of the vaccine was demonstrated. Further to Competent Authority approval deliberate release trials proceeded at the same location and confirmed the safety and efficacy of the vaccine.

3. What are the effects of the excreted GMO on:

- **a. human health, direct and indirect;**
- **b. soil dwelling natural *R. equi;***
- **c. the environment and the ensuing effect on animals, birds and wildlife, flora, pollinating insects;**
- **d. the ecosystem of the Rivers Brosna and Shannon where it is presumed the GMO will end up;**
- **e. Farming in the area and farm income should there be adverse effects.**

Agency's Response

Human health

Although persons handling horses are very frequently exposed to this pathogen human *R. equi* infections are extremely rare and occur almost exclusively in immunocompromised individuals.

The vaccine strain *R. equi* RG2837 carries the VapA$^+$ virulence plasmid which renders the bacterium equine specific. While the vaccine strain is pathogenic for immunocompromised persons, it has been shown to be less able to survive in human macrophages[10]. It can be expected that the risk for immunocompromised persons is at most equal but more likely less, compared to the wild type *R. equi*.

[10] Attenuation was confirmed in a human macrophage cell line

R. equi infection in foals can be fatal if they are not treated quickly. Treatment involves the administration of antibiotics and it is costly. Furthermore the antibiotics used are potent human antibiotics and their use may lead to the emergence of resistant strains of *R. equi*. This highlights the need for alternative strategies to antibiotic treatment such as vaccination.

As already outlined, a number of measures have been implemented under condition 6 to inform staff of the health hazards associated with *R. equi* bacterium and in particular in relation to immunocompromised persons, to minimise the number of people coming into contact with the GM vaccine, the area in which it will be used and the vaccinated foals

Soil dwelling natural R.equi

Wild type *R. equi* is ubiquitous in the Irish environment and is endemic on Belmont stud farm. The *R. equi* vaccine strain is a deletion mutant and so will not introduce any foreign genes over and above those already present in the environment. Furthermore genes were deleted from the chromosome rather than from mobile plasmids.

The $VapA^+$ virulence plasmid carried by the *R. equi* vaccine strain may be transmitted to plasmid free (non-pathogenic) *R. equi* strains rendering them pathogenic. However, this would present no greater risk than exposure to naturally occurring pathogenic strains. Furthermore, the transfer of virulence plasmids would not entail the transfer of foreign genetic material.

Both the GM vaccine strain and the wild type *R. equi* strain can survive for some time in the environment but the vaccine strain will not survive for longer than the wild type strain.

c. The environment and the ensuing effect on animals, birds and wildlife, flora, pollinating insects

d. the ecosystem of the Rivers Brosna and Shannon where it is presumed the GMO will end up.

For reasons already outlined the attenuated vaccine strain is expected to behave the same as the wild type strain in the environment and is not expected to cause any greater environmental impact.

The vaccine strain is an attenuated strain in that it is less able to survive and reproduce in macrophages and is thereby incapable of causing illness in foals rather it imparts protective immunity. The vaccine strain will have no competitive advantage outside the host since macrophages do not occur in the environment. It will however survive in the environment for as long as the wild type strain. Studies were carried out by the notifier to demonstrate safety of the *R. equi* vaccine strain in chickens, rats, mice, pigs and calves.

The vaccine strain of may however transmit its virulence plasmid to plasmid-free *R. equi* strains by conjugation. This may convert non-pathogenic indigenous *R. equi* populations present in the intestinal tract of foals or in soil into pathogenic ones. However, this would pose no greater risk than exposure to naturally occurring pathogenic strains and does not involve the transfer of foreign genetic

elements. This issue is further dealt with by Professor Meijer's in his expert report (Appendix 2).

e. Farming in the area and farm income should there be adverse effects

Agency's response

This trial is taking place on a stud farm where wild type *R. equi* is already endemic.

Cattle are maintained on farms adjacent to the release site. Badgers, foxes, rabbits and rodents occur in the surrounding countryside. The *R. equi* vaccine strain carries the VapA$^+$ virulence plasmid which renders the vaccine strain specific to horses. Given this specificity, it is expected that the *R. equi* vaccine strain would be unable to cause disease in other animal species. Studies were carried out by the notifier to demonstrate that the *R. equi* vaccine strain does not infect chickens, rats, mice, pigs or calves. Furthermore, mares visiting the stud for natural breeding with a stallion will not be permitted during the vaccination programme.

4. What are the long-term, cumulative and unanticipated effects of the release,

Agency's response

The GM vaccine strain is a clean deletion mutant into which no foreign DNA has been inserted with the exception of 12 nucleotides left over from the vector used to construct the vaccine strain. Therefore there is no risk of introducing new genetic elements into the environment. Persistence of the vaccine strain in soil and water samples has been shown to be comparable to that of the wild type strain.

The vaccine strain has lost its pathogenicity, it can therefore no longer survive and reproduce in macrophages in the host but it can impart immunity against *R. equi*. Attenuation of the vaccine strain in foals as well as safety in mice, rats, chickens, pigs and calves were demonstrated by the notifier.

The vaccine strain may transfer its virulence plasmid to a non-pathogenic wild type *R. equi* strain thereby making the wild type strain pathogenic, however, this does not involve the transfer of foreign genetic elements and would pose no more risk than exposure to naturally occurring pathogenic strains.

The expert advice[11] received by the Agency (Appendix 2) has indicated that the vaccine strain only poses a negligible risk to the environment or to human and animal health.

5. What are the potential adverse effects of the GM vaccine likely to be and what counteractive / emergency / mitigating measures will be taken

Agency's response

The adverse effects that might occur as a result of the administration / shedding of the GM vaccine strain of *R. equi* are negligible relative to those caused by the wild type *R. equi*.

[11] From Professor Wim Meijer, UCD.

6. A number of legal concerns were expressed

a. Liability – with whom would liability rest if there a problem arose with this trial

Agency's response

The Environmental Liability Regulations S.I. No 547 of 2008 defines environmental damage under three categories of which land damage is probably be most applicable to the scenario surrounding this proposed trial. 'Land damage' is defined as "any contamination that creates a significant risk of human health being adversely affected as a result of the direct or indirect introduction in or under the land of substances, preparations, organisms or micro-organisms". Where 'land damage' is deemed to have occurred, the operator is liable under the aforementioned Regulations. However the overall risk to human health and the environment is negligible from this proposed field trial.

b. Is the GM R.equi strain patented?

Agency's response

Yes, however, neither the patent nor the patent holder is within the Agency's remit.

c. GM products are not exempt from the legal obligations Cartagena Protocol on Biosafety

Agency's response

The Cartagena Protocol on Biosafety to the Convention on Biological Diversity is an international agreement which aims to ensure the safe handling, transport and use of living modified organisms (LMOs) resulting from modern biotechnology that may have adverse effects on biological diversity, taking also into account risks to human health. The GMO (Transboundary Movement) Regulations 2003 give effect to the Regulation 1946/2003 on transboundary movements of GMOs and the Cartagena Protocol and it constitutes a system for notifying and exchanging information on the transboundary movements of GMOs to third countries, outside the EU. This GMM originated within the EU and the trial is taking place within the EU therefore transboundary movement has no relevance in this instance.

Preamble 13 of Regulation (EC) No 1946/2003 on the transboundary movements of GMOs states as follows "According to the Protocol, the Community may apply its domestic legislation in respect of the movements of GMOs within its customs territory"

7. This application is in contravention of the Aarhus convention which states that people are entitled to have immediate access to information about actions which may affect their environment (articles 3, 4 and 6).

Agency's response

The Aarhus Convention was ratified by Ireland on 12 June 2012. Despite recent ratification, according to DECLG (with whom the Agency consulted on this topic), all Environmental legislation was heretofore in compliance with the requirements of the Convention. In addition, in accordance with the GMO (Deliberate Release) Regulations, S.I. No. 500 of 2003, organisations which in the opinion of the Agency are concerned with environmental protection are served with 2 representatives on the GMO Advisory Committee. A broad range of NGOs were consulted with regard to these nominations.

Furthermore, in accordance with Article 15 of the Regulations, the notifier published a notice in the Farmer's Journal on 5th January 2013, informing members of the public of the proposed trial and the provision to submit representations to the EPA within a 28 day period. However, as already detailed under the 'Making of Representations' in the previous section, the public had access to the full notification (less confidential information) for 11 days only as opposed to the required 28 days. In an effort to rectify this and on foot of legal advice, any person who had made a request for information or who had submitted a representation (valid or invalid) to the Agency within the 28 day period commencing 5 January, were given a further 28 days to consider the application (less confidential information) and amend their submission or make a new submission. The Agency received 2 new representations from persons who had not previously made submissions during this additional 28 day period.

The EPA has published full details of the proposed trial on its webpage.

8. Concern was expressed that the modified organisms present in the vaccine will combine with naturally present *R. equi* bacterial population in the soil and further mutate causing widespread contamination.

Agency's response

The vaccine strain of *R. equi* is a deletion mutant. Four genes at two loci, *ipdAB1* and *ipdAB2*, were deleted from the chromosome of *R. equi* RE1 to yield R. equi RG2837. There was no insertion of foreign DNA.

Recombination between the wild type *R. equi* strain and the *R. equi* vaccine strain would require the taking up of all four genes and their integration in the right place on the chromosome and in the correct orientation which theoretically is almost impossible. In any case, the end result would be the recreation of the wild type *R. equi* strain with a similar virulence as the wild type *R. equi* strains that are already present in high abundance in the environment and in the gut.

9. If there is indeed a risk of shed organisms contaminating adjacent waterways via groundwater escape from the Belmont site then how can the removal of straw and faeces be seen as an effective post release treatment when contaminated liquids are able to reach the surrounding drainage systems?

Agency's response

There is no identified risk of shed *R. equi* RG2837 contaminating adjacent waterways. The wild type parent strain of *R. equi* is a soil organism and is ubiquitous in the environment and can be found in diverse environments ranging from soil and ground water to insect guts and plant surfaces. The vaccine strain is a deletion mutant to which no foreign genes have been introduced. It is not expected to have any increased fitness or invasiveness in the environment over the existing wild type strain rather there is no difference in survivability between vaccine strain and wild type *R. equi* strain.

10. A lot of information in the application is deemed confidential, on what grounds were such decisions made?

Agency's response

Article 10 of the Regulations makes provision for confidential information.

Further to receipt of a request for confidentiality, OCLR assesses the information, considers whether it should be treated as confidential and whether the information is necessary to the processing of the application. Under the EMA centralised procedure for the placing of human and veterinary medicines on the market, all information is confidential until such time as the product has been approved and placed on the market. This is in complete contrast to Directive 2001/18/EC (Part B) under which there is provision for confidential information but it is kept to a minimum.

In this instance, the notifier requested that study reports be kept confidential on the grounds that:

- they contain detailed information on animal experiments, their location and details of the personnel involved. According to the applicant, release of this information could pose a security risk to the people involved and the locations where the studies are performed;
- at present, there are no vaccines on the market against *R. equi* in foals. Release of research data could provide a competitive advantage to other companies and in turn affect Intervet International's competitive position.

The Agency has no remit for the following issues raised in representations: business and academia in science; corruption in science; bending science; fragmentation of science; limitations of a specialist; source of relevant evidence; biotech blinkers; *in vivo/in vitro* issues and molecular life sciences; ECJ and science; and, reputational aspects.

A number of representations queried the authority of the Agency to make decisions in respect of the performance of GM veterinary trials, to accept finances with particular reference to the requirement to pay a €10 in order to make a representation and how this money will be used.

The EPA is an independent public body established under the Environmental Protection Agency Act, 1992 the Agency's mandate for the regulation of genetically modified organisms is stipulated under section 111 of the EPA Act. The requirement to pay a €10 fee for the submission of a representation is similarly set out in legislation under Articles 16 and 48 of the GMO (Deliberate Release) Regulations S.I. No 500 of 2003. The money collected will contribute to the self-financing of the Agency.

Review of the notification by the EPA and external consultation

The Agency's review of the notification involved both an internal and external review.

External Review

The GMO Advisory Committee

The Agency held a meeting of the GMO Advisory Committee (GMO AC) on the 7th March 2013. During this meeting committee members were given full access to confidential information submitted in connection with this application which was subsequently discussed.

Five GMO AC members made written submissions and of the five, two members were unable to attend the meeting.

One Committee member recommended the acceptance of this application.

A second GMO AC member did not identify any concerns in relation to the use of this GMO in a veterinary trial. This committee member did however say that it was not clear how the chromosomal deletion had been achieved and if the remaining chromosomal DNA had been sequenced to determine the occurrence of deletions other than those intended. This topic was also raised at the GMO AC meeting by yet another Committee member.

Agency's response
Clarification was sought from the notifier who duly provided a schematic aligning the deleted sequences with the wild type control sequence for comparison.

A third GMO AC member queried the design and scientific robustness of the experiments dealing with *R. equi* survival in macrophages and in the environment. According to this member, no information was provided on the number of replications carried out and no information was included on the detection limits of the microbiology tests reported. This member suggested that all these issues should be addressed by the notifier before a decision could be reached on this application.
Agency's response
Again these issues were subsequently addressed to the notifier who in essence responded that many more macrophage survival tests had been performed in the past (with some being provided in other annexes) and while not statistically analysed, they always showed the same pattern of reduced survival for the vaccine strain compared to the wild type parent strain. The detection limits of the microbiology tests were also answered to the satisfaction of the Agency.

A fourth Committee member highlighted a number of aspects many of which have already been addressed in this report. This Committee member also drew attention to the lack of direct correlation between survival in macrophages and human pathogenicity.
Agency's response
The notifier responded that the performance of experiments in immunocompromised humans is not feasible. The inability of the vaccine strain to cause disease in immunocompromised humans (compared to wild type *R. equi* which is ubiquitous in the environment) is based on the fact that the vaccine strain is attenuated for foals and it is less able to survive in human macrophages.

The fifth AC member stated that there was insufficient information on the notification to determine the extent of the hazards to human health and environment health. In particular, attention was drawn to the transfer of the virulence plasmid in *Rhodococcus equi* and queried the experimental evidence that conjugation between *R. equi* RG2837 and other *R. equi* strains were improbable.
Agency's response
The notifier and the expert opinion provided to the Agency both acknowledge the transmission of virulence plasmids from the vaccine strain to plasmid free (non-

pathogenic) *R. equi* strains thereby rendering them pathogenic. However this would pose no more risk than exposure to naturally occurring pathogenic strains.

Such issues as Influence and Regulatory Capture as raised by this GMO AC member are outside the Agency's remit.

During the course of the GMO AC meeting, a number of issues primarily relating to the safety of the vaccine in other animals (most notably small sample sizes and inadequate follow-up on an animal's wellbeing) and the survival of *R. equi* in macrophages were identified as requiring further clarification.

Agency's response

The notifier agreed that a group of 5 animals is a minimal group size but is sufficient for clear serious rapidly developing effects. Larger groups would be required for rare effects. The notifier indicated that it would be difficult if not impossible to obtain permission form the Dutch ethical committee for large numbers of animals for this purpose. In addition *R. equi* RG2837 has been shown to be safe for the foal (the most sensitive species) making it unlikely to observe adverse reactions in other species.

Two Committee members expressed concern about these proposed trials proceeding saying it was too soon to have deliberate release trials involving GM vaccines. Rather, further investigations were warranted under contained use before moving on to deliberate release. There was general agreement among Committee members that the monitoring plan should be elaborated upon, in particular, in terms of the nature of the monitoring to be carried out and the area to be monitored.

Agency's response

The extent of monitoring to be carried out was addressed by the notifier in response to the Agency's request for further information. It should be noted that this vaccine was tested under contained use in the NL prior to the performance of veterinary trials under deliberate release and no adverse effects were identified.

It was also stated during the AC meeting that vaccinated foals should not be moved out of the release site until such time as they were tested for shedding and the non-shedding of the *R. equi* vaccine strain confirmed. This has been conditioned for under Condition 5.0.

Independent expert

The Agency sought the expert opinion of Professor Wim Meijer, a molecular microbiologist and Associate Professor in the School of Biomolecular and Biomedical Science at University College Dublin. Professor Meijer's research focusses on the virulence mechanisms of *Rhodococcus equi.*

Professor Meijer reviewed the full application and provided a report in which he stated that the *R. equi* vaccine strain poses only a negligible risk to the environment or to human or animal health. He did indicate that it may transmit its virulence plasmid (carrying no genetic modification) to plasmid free *R. equi* strains occurring in the foal gut or in the soil. While this may convert the recipient strain to a virulent strain, it would pose no more risk than exposure to naturally occurring virulent strains. Furthermore there would be no transfer of foreign genetic elements.

Professor Meijer also stated in his report that the GMM is safe, the measures employed to avoid and/or minimise the spread of the GMM beyond designated areas of use are sufficient and no additional monitoring is required.

With regard to the risks this GMM poses to human health, Professor Meijer stated that despite the frequent exposure of persons handling horses to this pathogen, human *R. equi* infections are extremely rare and occur almost exclusively in immunocompromised individuals.

Dr Meijer's report is provided in Appendix 2.

Other regulatory bodies and Government Departments

The Agency also consulted the following regarding the proposed deliberate release:

- Department of Agriculture, Food and the Marine (DAFM);
- Irish Medicines Board (IMB) (for information).

Department of Agriculture, Food and the Marine

The Agency consulted with the Veterinary Medicines section of DAFM who in turn informed the Agency in writing that no issues had been raised in relation to the GM aspect of the trial vaccine.

Consultation with other EU member states

In accordance with Article 18(1)(b) of the Regulations the Agency submitted the SNIF to the Commission within 30 days of receipt of the notification. The Commission published the SNIF to all other EU member states for comment. The Agency did not receive any comments or observations from other member states.

Internal review

As part of the Agency's internal review the Agency visited the site of the proposed deliberate release at Belmont Stud Farm, Co Offaly on 11th March 2013. The Agency met with the owner of the site as well as veterinary staff from MSD Animal Health Ireland and MSD Animal Health The Netherlands.

It was confirmed during the course of this visit that vaccinated foals will not be moved from the release site until such time as a 'clean' rectal swab for the *R. equi* vaccine strain is recovered. This test will be done by PCR in the Netherlands and will take up to one week to perform.

The GM vaccine will be administered by a vet and veterinary staff from MSD Animal Health Ireland and The Netherlands (whom the Agency met during the course of this site visit) will visit periodically to check on the administrative aspects of the trial, i.e., paperwork, reporting, etc.

The Agency was satisfied with the proposed arrangements.

In addition to the site inspection, the EPA has reviewed the notification, the additional information submitted by the notifier and the submissions made by members of the public and this Inspector's Report is the product of that internal review.

Conclusions

After examining the notification supplied in the notification under Article 14 of the GMO (Deliberate Release) Regulations S.I. No 500 of 2003 and the further information provided by the notifier in response to a request from the Agency under article 19(1), OCLR conclude that this notification is in compliance with the aforementioned Regulations.

The objective of this trial is to check the efficacy of a GM vaccine against *R. equi* in foals under field conditions. Wild type *R. equi* is already ubiquitous in the environment and endemic on Belmont Stud Farm where it is proposed that this trial will take place.

The vaccine strain of *R. equi* is a deletion mutant. Four genes from two loci on the bacterial chromosome were deleted with the addition of no foreign genetic material. These deletions render the vaccine strain of *R. equi* unable to grow and survive in macrophages in the foal's lung (which is necessary in order to bring about pneumonia). Therefore, the vaccine strain is an attenuated strain and serves to impart immunity to the foal.

The vaccine strain will be shed in the foal's faeces for 1 – 2 days after each vaccination (peak shedding) and reduced shedding will continue for a period thereafter. Foals actively shedding *R. equi* RG2837 will not be moved from the release site until such time as shedding has ceased. While the vaccine strain is attenuated within the foal, it will behave as per the wild type strain in the environment. Since the vaccine strain does not contain foreign DNA there is no risk of introducing new genetic elements into the environment.

The vaccine strain will carry the VapA^{+} virulence plasmid which is equine specific.

VapA^{+} virulence plasmids may be transmitted to non-pathogenic wild type strains of *R. equi* by conjugation thereby rendering them pathogenic. However, these strains would pose no greater risk than naturally occurring pathogenic strain which are already ubiquitous in the environment.

The attenuated *R. equi* vaccine strain could cause infections in immunocompromised humans. However the vaccine strain has been shown to be less able to survive in human macrophages. It can be expected that the risk for immunocompromised persons is at most equal but more likely less compared to the wild type *R. equi*. Furthermore, condition 6 limits the accessibility of immunocompromised persons to the proposed trial site.

Fee payable to the EPA

The appropriate fee of €3,000 as outlined in the Part IV of the GMO (Deliberate Release) Regulations (S.I. 500 of 2003) has been paid in respect of a notification for a proposed deliberate release for purposes other than placing on the market.

Recommendation

I am satisfied that on the basis of the review carried out and in particular, on the basis of the expert opinion available to the Agency that the risks posed to the environment and human health (general population) by the deliberate release of this GMM are negligible.

On this basis I recommend that consent be granted to Intervet International B.V. to conduct a clinical trial under Part B of the GMO (Deliberate Release) Regulations to test the efficacy of a GM vaccine 'Equilis RhodE' at Belmont Stud Farm, Belmont, Co Offaly from the date of grant of consent conditions by the Agency to 30 September 2016 subject to the conditions set out in the attached draft Consent Conditions.

Signed: _____ Date: 04/04/2013.

Bernie Murray
Inspector
Office of Climate Licensing & Resource Use

APPENDIX 1: Aerial map of Belmont Stud Farm

Department of
Agriculture,
Fisheries and Food
An Roinn
Talmhaíochta,
Iascaigh agus Bia

For Single Payment Scheme Purposes only

Scale: 1:5000

Name: Andrea Eller
Address:
Belmont House Stud
Belmont
Co Offaly

Townland Code: S14901
Townland Name:
BELLMOUNT or LISDERG

Parcel	Digitised	Ref	Claimed
S14901062	9.97 Ha	9.10 Ha	8.50 Ha
S14901063	1.11 Ha	0.00 Ha	0.00 Ha
S14901074	7.22 Ha	7.25 Ha	7.25 Ha

Ortho Used: COL_ORTHO_2009LF

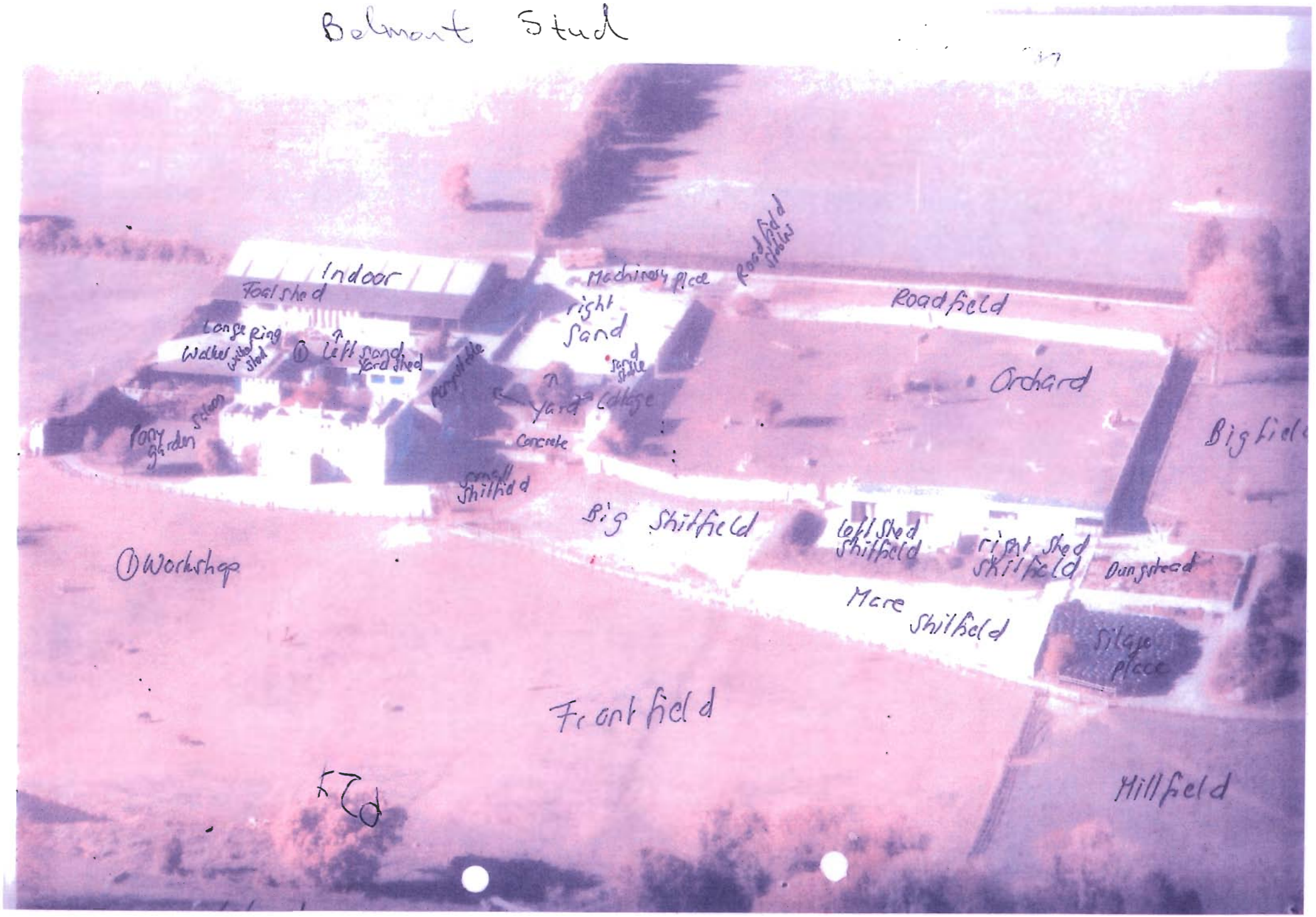
* Areas shown above are gross areas in hectares. Deductions should be made for non-agricultural areas in Single Payment Scheme Applications

** Validation checks may have altered the gross area of some parcels. You should therefore examine the areas of individual parcels carefully.

S 1 4 9 0 6 3 9

Fri Jan 21 00:03:59 GMT 2011
S1490639 1 of 2

Belmont Stud



APPENDIX 2: Expert opinion of Professor Wim Meijer

UCD School of Biomolecular and Biomedical Science

Scoil na hEolaíochta Bithmhóilínigh agus an Bithleighis UCD

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An Coláiste Ollscoile, Baile Átha Cliath,
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wim.meijer@ucd.ie
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Dr. Tom McLoughlin
Environmental Licensing Programme
Office of Climate, Licensing & Resource Use
Environmental Protection Agency
PO Box 3000
Johnstown Castle Estate
Co. Wexford

11 March 2013

Re: expert opinion on Rhodococcus equi

Dear Tom,

I have read the application in detail. Please find enclosed my report answering your specific questions as send to me by Bernadette in her email of 18 February.

Please let me know if you require additional information or if you would like me to expand on any particular issue in the report.

Best wishes,

Wim G. Meijer, PhD
Associate Professor and Head of Microbiology

Report relating to release of Rhodococcus equi strain RG2837 as a vaccine against foal pneumonia.

Is the GMM safe? Is this GM vaccine and in particular the GMM, through shedding, likely to cause an adverse effect

a. **on the environment**
b. **on human and/or animal health**

whether direct or indirect, immediate or delayed or through the spread of the GMM in the environment, the uptake of genetic material from other organisms in the soil or gut?

Wild type virulent *R. equi* strains harbouring a VapA-type virulence plasmid are major pathogen of young foals, which shed large numbers in their manure. Research has shown that these *R. equi* strains are ubiquitously present in Ireland. However, although persons handling horses are very frequently exposed to this pathogen, human *R. equi* infections are extremely rare and occur almost exclusively in immuno-suppressed individuals.

The vaccine strain *R. equi* RG2837 differs from the wild type parent strain in that four genes in two loci, *ipdAB1* and *ipdAB2*, were deleted from the chromosome. With exception of 12 nucleotides, the mutagenesis procedure did not introduce any foreign DNA into the vaccine strain. These deletions prevent cholesterol metabolism, rendering the vaccine strain unable to grow within macrophages. Attenuation of the vaccine strain in foals, as well as its safety in mice, rats, chickens and pigs were demonstrated by the applicants. They also convincingly demonstrated that otherwise the vaccine strain has identical properties as the wild type parent strain including survival in the environment.

Since the vaccine strain does not contain foreign DNA, there is no risk of introducing new genetic elements, such as antibiotic resistance markers, into the environment. Attenuation has been achieved through deletion of two loci on the chromosome. It therefore is extremely unlikely that the attenuated vaccine strain will revert to a virulent wild type phenotype.

The virulence plasmid of the vaccine strain is identical to that of the parent strain and to that of the well studied strain *R. equi* 103S. It has recently been demonstrated that the virulence plasmid can be transferred efficiently by conjugation to an avirulent recipient, rendering the latter virulent (Tripathi et al, 2012). Although the vaccine strain is attenuated, it may transfer its virulence plasmid to an avirulent wild type *R. equi* strain lacking a virulence plasmid.

Conclusion

In my view the vaccine strain itself poses only a negligible risk to the environment or to human and animal health. It may however transmit its virulence plasmid (which is not genetically modified) to plasmid-free *R. equi* strains. In theory, this may convert avirulent indigenous *R. equi* populations present in the intestinal tract of foals or in soil into a virulent one. However, this would pose no more risk than exposure to naturally occurring virulent strains, and does not involve transfer of foreign genetic elements. The containment methods proposed in the application are sufficient to

prevent the spread of any virulent *R. equi* that may arise from transfer of the virulence plasmid from the vaccine strain to other naturally occurring *R. equi* strains.

In summary, in my view the GMM is safe.

In your opinion, is the Risk Assessment satisfactory?

Yes, the applicants thoroughly considered the risks associated with release of the GMM, any potential adverse outcomes as well as measures to prevent or contain these.

Should the Agency impose any particular conditions or consider any particular monitoring to be carried out by the applicant.

The measures described in the application under section 1.1.5.B. 'Control of the Release' are in my view sufficient. No additional conditions or monitoring is required.

Reference

V. N. Tripathi, W. C. Harding, J. M. Willingham-Lane, and M. K. Hondalus. Conjugal transfer of a virulence plasmid in the opportunistic intracellular actinomycete *Rhodococcus equi*. *J.Bacteriol.* 194:6790-6801, 2012.