PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Netherlands
(b)	Notification number	B/NL/09/004
(c)	Date of acknowledgement of notification	27/09/2010
(d)	Title of the project	A non-pathogenic *Rhodococcus equi* strain as vaccine in horses.
(e)	Proposed period of release	From Jan 2011 until product license

2. Notifier

Name of institution or company: Intervet International bv, Boxmeer, The Netherlands

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)
RNA virus		(.)
DNA virus		(.)
bacterium		(x)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)
Deletion mutant of *Rhodococcus equi* strain RG2837

(c) Genetic stability – according to Annex IIIa, II, A(10)
It is a stable unmarked deletion mutant of *Rhodococcus equi.*

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (x) No (.)

If yes, insert the country code(s) DE, permit pending

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (.) No (x)

If yes:

- Member State of notification …
- Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (x)

If yes:

- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.

Rhodococcus equi is a common soil bacterium that also can colonize the gut and nasal passages of animals, especially herbivores, and is the cause of severe pneumonia in foals. The GMO (vaccine strain) is an unmarked deletion mutant of *Rhodococcus equi*. Because of this deletion the bacterium is unable to survive in macrophages (in contrast to the wild type) and therefore unable to cause pneumonia in foals (in contrast to the wild type). Outside the animal host the bacterium shows a similar survival to the wild type. Environmental impact of release of the GMO is judged effectively zero.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (x)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)

- other animal (.)
  (specify phylum, class) …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	…
(iii)	species	*Rhodococcus equi*
(iv)	subspecies	…
(v)	strain	RE1
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	…

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (x) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:
The bacterium occurs world-wide in soil and surface water, especially where herbivores graze. In these animals it colonizes nasal cavities and gut. In foals colonization of airways can lead to pneumonia.

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (.)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (.)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (X)

soil, free-living (X)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify colonizes nasal cavities and gut of animals, especially herbivores

(b) If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques
Isolation on selective agar and PCR

(b) Identification techniques
Bacteriological determination and PCR

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment
Yes (x) No (.)
If yes, specify
EC class 2 organism (EC 2000/54/EG)

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (x) No (.) Not known (.)

If yes:

(a) to which of the following organisms:

humans (x) only immunocompromised humans
animals (x) pneumonia in foals
plants (.)
other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

The live wildtype *Rhodococcus equi* can cause pneumonia in foals and can cause infections in immunocompromised humans (i.e. AIDS patients).
R. equi primarily causes infections in foals. *R equi* rarely infects immunocompetent humans. During infection in foals *R equi* acts as an intracellular bacterium and survives within macrophages and eventually destroys them. Experimental data suggest that *R equi* is capable of inhibiting oxidative bactericidal functions of polymorphonuclear cells. Electron microscopy of *R equi* in equine macrophages demonstrates that the organisms appear to avoid being killed by interfering with phagosome-lysosome fusion.
Most of the information about the pathogenesis of R equi infections is derived from animal isolates. However, the infection in humans seems to differ from that in foals. A 15- to 17-kd virulence-associated protein antigen (VapA), is highly associated with virulence in foals. Nearly all isolates from pigs have a 20-kd virulence-associated

protein antigen (VapB). In human beings, only about 20-25% of isolates have been reported to express VapA, or VapB. The rest does have either Vap encoding gene. There are no reports about toxigenicity, allergenicity or vectors.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Depending on conditions 30 min to days

(b) Generation time in the ecosystem where the release will take place:
See previous

(c) Way of reproduction: ~~Sexual~~ .. Asexual: cell division

(c) Factors affecting reproduction: temperature, nutrients

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	

(b) relevant factors affecting survivability:
…

10. (a) Ways of dissemination
Wind (attached to dust particles), animals (nasal and or gut colonization).

(b) Factors affecting dissemination
Presence of herbivores, housing practices and weather conditions

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
Not applicable

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(.)
(ii)	deletion of genetic material	(x)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
Unable to survive in macrophages (in contrast to wildtype)

3. (a) Has a vector been used in the process of modification?
Yes (x) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) No (x)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid (.)
bacteriophage (.)
virus (.)
cosmid (.)
transposable element (.)
other, specify …

(b) Identity of the vector
…

(c) Host range of the vector
…

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (.) No (.)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (X)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert: Not applicable (deletion mutant).

(a) Composition of the insert
…

(b) Source of each constituent part of the insert
…

(c) Intended function of each constituent part of the insert in the GMO
…

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify …

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (.)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Complete name

(i) order and/or higher taxon (for animals) …
(ii) family name for plants …
(iii) genus …
(iv) species …
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (.)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (.) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (X)* No (X)** Not known (.)

*Yes: the GMO can no longer survive in macrophage. Invasion and growth of the alveolar macrophages is essential for the development of pneumonia.
**No: there appears to be no difference in survival in the environment.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X)* No (X)** Unknown (.)

*Yes: the GMO can no longer reproduce in macrophage. Invasion and growth of the alveolar macrophages is essential for the development of pneumonia.
**No: there appears to be no difference in growth in the environment.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X)* No (X)** Not known (.)

*Yes: as the GMO is not able to grow in the lungs there will be less dissemination.
**No: there appears to be no difference in growth in the gut and therefore rectal excretion will be similar.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (X) No (.) Not known (.)

The GMO can not survive and grow in alveolar macrophage, the site of infection that is central in the development of R. equi pneumonia in foals.

2. Genetic stability of the genetically modified organism
See section A.3.c

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

The GMO can not be monitored directly in the environment. Indirect monitoring can be done by taking samples and plate them out on selective agar. Positive identification will follow from R. equi and GMO specific PCR's.

(b) Techniques used to identify the GMO
Selective agar and PCR's based on the genome region that has been modified.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected):

Study the efficacy of the vaccine under field conditions

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (.) No (x)
If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
The vaccine will be tested in foals on farm

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
No

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Rodents and birds can come into contact with pasture.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Vaccine dose consists of approximately 0.5 -9 x 10^{10} CFU per dose

(b) Duration of the operation:
Field trials on different locations; duration per location approximately 6-12 month.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Animals are physically contained in stables and/or (fenced) pasture

5. Short description of average environmental conditions (weather, temperature, etc.)

Duration of field trials will be 6-12 months per location under weather conditions as usually found in The Netherlands

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

Non available

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	
Kingdom:		Animals
Phylum:		Vertebrates
Class:		Mammals
(ii)	family name	Equidae
(iii)	genus	*Equus*
(iv)	species	*ferus*
(v)	subspecies	*caballus*
(vi)	strain	…
(vii)	cultivar/breeding line	all breeds
(viii)	pathovar	…
(ix)	common name	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Vaccine strain will transiently be present in the intestine and the lung and interact with the local lymph nodes and thereby inducing a protective immune response

3. Any other potentially significant interactions with other organisms in the environment
Outside the animal host the vaccine strain will behave similar to the wild type.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
…

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
In the soil (pasture) where the horses graze.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

Not applicable, non-pathogenic vaccine strain.

(i) order and/or higher taxon (for animals) …

(ii) family name for plants …
(iii) genus …
(iv) species …
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
unlikely

(b) from other organisms to the GMO:
Unlikely

(c) likely consequences of gene transfer:
Consequences of genes transfer will be unlikely. Occurrence of gene transfer is not more likely than for wildtype R. equi.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
Not available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Isolation on selective agar and further identification by PCR.

2. Methods for monitoring ecosystem effects
The protocol contains a description of the monitoring and data system concerning the animal and its immediate environment.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable, it is a deletion mutant

4. Size of the monitoring area (m^2)
Horse farm with pasture i.e. approx hundred m^2

5. Duration of the monitoring
6 month to 1 year

6. Frequency of the monitoring

Shortly after vaccination the animals will be monitored daily, later this may become less frequently but at least once a week.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

No post-release treatment necessary; it is an attenuated deletion mutant.

2. Post-release treatment of the GMOs

Not applicable (see above).

3. (a) Type and amount of waste generated

Not applicable (see above).

3. (b) Treatment of waste

Not applicable (see above).

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

Controlling spread is not necessary since it is an attenuated deletion mutant, there is not more risk then the already present wildtype *Rhodococcus equi.* See section J4

2. Methods for removal of the GMO(s) of the areas potentially affected

Not applicable (see above).

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Not applicable (see above).

4. Plans for protecting human health and the environment in the event of an undesirable effect

Despite the negligible risk related to the use of the vaccine strain RG2837, an emergency plan has been established in which three operating phases are implemented.

1. Alert phase
Any observation that cannot be related to normal post vaccination reactions must be reported to the investigator, the veterinarian and the MVF.

2. Investigation phase
Appropriate samples are collected and sent to the laboratory for isolation and identification. If present, the diseased animals will be treated with antibiotics. Dead animals will be

destroyed. In the unlikely case that humans are affected they also will be treated with antibiotics.

3. Action phase

The study will be cancelled; stables, feces, soil and straw will be destroyed or disinfected.