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/10/004
(c)	Date of acknowledgement of notification	19/07/2011
(d)	Title of the project	Non-pathogenic *Mannheimia haemolytica* and *Pasteurella multocida*
(e)	Proposed period of release	From Sep 2011

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 mutants of *Mannheimia haemolytica* strain NADC-D153 and *Pasteurella multocida* strain NADC 1062.

(c) Genetic stability – according to Annex IIIa, II, A(10)
Stable unmarked deletion mutants of *Mannheimia haemolytica* and *Pasteurella multocida*

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

Yes (.) No (x)

If yes, insert the country code(s)

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 (x) No (.)

If yes:

- Member State of notification released in the US
- Notification number Approval letter from USDA, Environmental Assessment and Finding of No Significant Impact; July 2008

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

Mannheimia haemolytica and *Pasteurella multocida* are commensal bacteria found in the upper respiratory tract of ruminants. However, after periods of stress (e.g. viral infection, overcrowding, sudden weather changes, transport etc.) they act as an opportunistic pathogen and can strongly proliferate and cause pneumonia. The vaccine strains (GMO's) are unmarked deletion mutants of *Mannheimia haemolytica* and *Pasteurella multocida*. The deletions were designed to make the vaccine strains strongly attenuated and unable to cause disease. The safety and attenuation of the vaccine strains has been demonstrated in mice, calves, rabbits, chickens, goats and sheep. Although limited shedding after intranasal vaccination may occur, the vaccine strains are unable to grow/survive outside the animal host. Taken together the safety for animals, the limited survival capacity (in and outside animals) and the fact that the vaccine strains are unmarked deletion mutants, environmental impact of release of these GMO's 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	*Mannheimia haemolytica*
(iv)	subspecies	…
(v)	strain	NADC-D153
(vi)	pathovar (biotype, ecotype, race, etc.)	A1
(vii)	common name	…

2. Name

(viii)	order and/or higher taxon (for animals)	…
(ix)	genus	…
(x)	species	*Pasteurella multocida*
(xi)	subspecies	…
(xii)	strain	NADC-1062
(xiii)	pathovar (biotype, ecotype, race, etc.)	A3
(xiv)	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:

Both bacteria occur world-wide in the upper respiratory tract of practically all ruminants. Outside the animal host they can not survive for a long time.

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	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	(X)

Natural habitat of both bacteria is upper respiratory tract of ruminants. They cannot survive for a long time outside the host.

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

5. (a) Detection techniques
Isolation on agar and/or 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
Both organisms are EC class 2 organisms (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	(.)
animals	(x) opportunistic secondary pathogens: pneumonia in ruminants
plants	(.)
other	(.)

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

Wild type *Mannheimia haemolytica* and *Pasteurella multocida* are opportunistic commensal bacteria that are present in low numbers in practically all (healthy) cattle world wide. After periods of stress (e.g. viral infection, transport, overcrowding, sudden weather changes etc) these bacteria (either alone or in combination) can strongly proliferate and cause pneumonia. Outside the animal host they can not survive for a long time.
Mannheimia haemolytica normally produces a leukotoxin which, together with the LPS, is regarded as a major virulence factor. The capsule of *Pasteurella multocida* serotype A, together with the LPS, is regarded as a major virulence factor for this species. *Mannheimia haemolytica* is restricted to ruminants but *Pasteurella multocida* serotype A has been associated with atrophic rhinitis in pigs, fowl cholera in poultry and respiratory disease in ruminants. However, most *Pasteurella multocida* isolates appear to be specialized for virulence in the host of isolation and do not appear to be able to cause disease in other species. The parent strain (NADC-1062) was isolated in the USA from lung tissue of a calf that died of pneumonia.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
in animal host ± 30 min

(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:
Immune status and/or stress status of host

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
Nose-nose contact, drinking bowls, aerosols and/or attached to dust particles (wind).

(b) Factors affecting dissemination
Animal density, housing practices, weather conditions, stress situations

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
Attenuated bacteria, less able to cause disease.

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: Not applicable

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 (.) Not known (.)
Specify: compared to the wild type, survivability in the animal is less

(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 (.) Unknown (.)
Specify: reproduction in animals is hampered because of the deletions.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (x) No (.) Not known (.)
Specify: because of the attenuation the bacteria are less able to colonise the respiratory tract and also less able to disseminate.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (x) No (.) Not known (.)
Specify because of the attenuation the bacteria are less able to cause pneumonia; on the contrary the GMO's induce protection against wild type infection.

2. Genetic stability of the genetically modified organism
Both are stable unmarked deletion mutants of *Mannheimia haemolytica* and *Pasteurella multocida*

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 bacteria do not grow/survive for a long time outside the animal host. Nose swabs can be sampled and inoculated on agar for isolation and/or tested directly in the PCR.

(b) Techniques used to identify the GMO
Bacterial determination and/or PCR.

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 (protection against the disease in calves) under field conditions and develop a registered vaccine for EU use.

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 calves on the Intervet animal farm.

(b) Size of the site (m^2): 100.000 m^2
(i) actual release site (m^2): 200 m^2 (stables)
(ii) wider release site (m^2): 100.000 m^2 (pasture and/or stables)

(c) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
The bacteria can only survive transiently in ruminants; during the studies there will be no contact with other ruminants.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Vaccine dose can vary from $10^5 – 10^{11}$ CFU

(b) Duration of the operation:
The field trials will be carried out during several years until licensing of the vaccine. Each trial will have a variable duration (1-12 months)

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

5. Short description of average environmental conditions (weather, temperature, etc.)
Duration of the field trials will be 1-12 month per trial under weather conditions as usually found in the Netherlands (average winter, average summer temp). Calves will be housed in Iglos and/or Stables and/or double fenced pasture.

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.
During field trials in different states of the US (involving several hundreds of animals) no problems were encountered. The vaccine strains appeared to be safe and efficacious (i.e. induced a protective immune response against P.m. en M.h)

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	Bovidae
(iii)	genus	Bos
(iv)	species	primigenius
(v)	subspecies	taurus
(vi)	strain	…
(vii)	cultivar/breeding line	different breeds
(viii)	pathovar	…
(ix)	common name	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Vaccine strains will transiently be present in the upper respiratory tract or in the muscle after intranasal or intramuscular vaccination, respectively. The vaccine strains will interact with the immune system and thereby inducing a protective immune response.

3. Any other potentially significant interactions with other organisms in the environment
The safety and attenuation of the vaccine strains has been demonstrated when applied to mice, calves, rabbits, chickens, goats and sheep. Although limited shedding after intranasal vaccination may occur, the vaccine strains are unable to grow/survive outside the animal host.

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.
The vaccine strains are attenuated and shown to be safe when applied to mice, calves, rabbits, chicken, goat and sheep. After subcutaneous vaccination the vaccine strain will not be shed. After intranasal vaccination the vaccine strain may (transiently) colonize the nasal passages and may be shed into the environment but the vaccine strain cannot grow/survive outside the animal host. In addition during the study the calves will not have contact to other animals.

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; The vaccine strains are attenuated and apathogenic. They were shown to be safe when applied to mice, calves, rabbits, chicken, goat and sheep. In addition they can not grow/survive outside the animal host.

(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:
Unlikely (unmarked deletion mutants); occurrence of genes transfer are not more likely than for wild type bacteria.

8. Give references to relevant results (if available) from studies of the behavior 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 agar and PCR

2. Methods for monitoring ecosystem effects
Seen the nature of the vaccine strains: unmarked deletion mutants, shown to be apathogenic for animals and unable to survive outside the animals, monitoring ecosystem effects is not necessary.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable, both are unmarked deletion mutants.

4. Size of the monitoring area (m^2)
Not applicable.

5. Duration of the monitoring
Not applicable.

6. Frequency of the monitoring
Not applicable

I. Information on post-release and waste treatment

1. Post-release treatment of the site
No post-release treatment necessary; both are attenuated unmarked deletion mutants unable to survive outside the animal host.

2. Post-release treatment of the GMOs
No post-release treatment; treatment is not necessary as both vaccine strains are attenuated unmarked deletion mutants unable to survive outside the animal host.

3. (a) Type and amount of waste generated
Faeces/straw, used syringes, vaccine vials, swab and blood samples.

3. (b) Treatment of waste
Faeces and straw: will be stored in a container and eventually spread over farmland; treatment is not necessary; both are attenuated unmarked deletion mutants unable to survive outside the animal host.
used syringes, vaccine vials, swab and blood samples: will transported to the ML-II lab of Intervet and disposed of according to ML-II standard procedures.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Although antibiotic treatment of animals and chemical disinfection of faeces and straw are possible, controlling spread is not necessary since both vaccine strains are attenuated, safe unmarked deletion mutants; there is no more risk than with the already present wild type bacteria. In addition the vaccine strains cannot survive outside the animal host.

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 *Mannheimia* and *Pasteurella* vaccine strains, 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 Environmental safety officer.

2. Investigation phase
Appropriate samples are collected and sent to the laboratory for isolation and identification. If vaccine strain is identified (from affected tissue), the diseased animals will be treated with antibiotics and/or euthanized. Dead animals will be incinerated. In the unlikely case that humans are infected they will be treated with antibiotics.

3. Action phase
The study will be cancelled; stables, feces, soil and straw will be incinerated or disinfected.