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Vectormune FP-LT+AE

Lyophilized suspension for injection in chickens

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

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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

Note to readers:
Vectormune FP-LT+AE is a live vector vaccine developed for the prevention of fowlpox (FP) and infectious laryngotracheitis (ILT) in chickens, associated, in the same freeze-drying presentation, with a conventional vaccine strain for the prevention of avian encephalomyelitis (AE). The active ingredient in the vaccine is a modified live FPV, expressing the gB and UL-32 genes of LTV and a live AE conventional vaccine strain.
In this document, only information concerning the vector vaccine strain (also referred to as Vectormune FP-LT) will be shared and discussed. A specific field trial request procedure will be launched with the Hungarian authorities (National Food Chain Safety Office, Directorate of Veterinary Medicinal Products), where in the submitted dossier specific information on the AE vaccine component will be included. This will allow the full assessment of the data in order to authorise the release of the vaccine for the regulatory field trials.

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	***Hungary***
(b)	Notification number	B/HU/17/03
(c)	Date of acknowledgement of notification	28/03/2017
(d)	Title of the project	***Vaccination of chickens with a combination vaccine against infectious LT, FP and AE diseases.***
(e)	Proposed period of release	***From 26/06/2017 to 31/08/2018***

2. Notifier

Name of institution or company:
Ceva-Phylaxia Co LTD.
H-1107 Szállás utca 5
Budapest, Hungary
Tel: (+36 1) 262 95 05
Email: zoltan.esztergomi@ceva.com

3. GMO characterization

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	**(X)**
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)

- insect (.)
- fish (.)
- other animal (.) specify phylum, class
-

(b) Identity of the GMO (genus and species)

Vectormune FP-LT is a modified Fowlpox virus expressing the gB and UL-32 genes of laryngotracheitis virus as well as the LacZ gene of E. coli. Fowlpox virus (FPV) is classified in the family Poxviridae in the genus Avipoxvirus. It is the active ingredient of Vectormune FP-LT, a live freeze dried vaccine for chickens against fowlpox and infectious laryngotracheitis.

(c) Genetic stability – according to Annex IIIa, II, A(10)

The parental FPV strain was genetically modified by homologue recombination to contain the gB gene of the US field strain 632 of LTV, the UL-32 gene of the Japanese virulent strain of LTV, NS175 and the LacZ gene from E. coli. The genetic stability of the GMO was evaluated and compared to that of the FPV parent strain.

<u>In chickens (in vivo)</u>: the GMO and the FPV parent strain were 'back passaged' in chickens five times. The results showed no adverse vaccine reactions or clinical signs for both the GMO and FPV parent strain 'back passage' groups, showing that the parent strain and the GMO did not revert to virulence after five passages in chickens.

<u>In vitro</u>: the GMO was also passaged in cell culture (chicken embryo fibroblasts or CEF) five times. Passaged GMO was compared to the non-passed GMO, to the homology plasmid used in construction of the GMO and the non passaged FPV parent strain. Results of the in vitro passages showed no genetic changes in the GMO.

<u>Comparison of passaged and non-passaged strains</u>:
Both the in vitro and in vivo passaged GMO were evaluated by comparing before and after the passages, both gene structure and protein expression. Results indicated that the GMO was genetically stable after both in vitro and in vivo passage and no unexpected differences were seen when compared to the genomic FPV parent DNA, genomic Vectormune FP-LT MSV DNA and the homology plasmid. For this analysis, Southern Blot, Western Blot, PCR, sequencing and Black plaque assays were carried out.

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 (X) No ( )

If yes:

- Member State of notification ES
- Notification number B/ES/13/22 dated 21 April 2014

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 …
- Notification number (see 2 attachments)

Two vaccines containing the same GMO strain (Vectormune FP-LT+AE and Vectormune FP-LT) are currently authorized and marketed in the USA, Argentina, Bangladesh, Bolivia, Brazil, China, Colombia, Costa Rica, Ecuador, Egypt, Kuwait, Mexico, Pakistan, Peru, Philippines, Russia, Saudi Arabia, South Africa, Thailand and Ukraine.

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

The outcome of the human and environment risk assessments show that the risk to public health is negligible for Vectormune FP-LT. Human exposure will be limited to persons administering this vaccine or handling vaccinated chickens. Fowl poxvirus (FPV) is known not to be of public health significance and no known infection in humans has been reported.
The GMO is safe in the target species (chickens) and does not spread from vaccinated chickens (target species) to other chickens. It has a narrow host range and its capacity to disseminate in the environment is limited. Vaccine spillage at time of vaccination can be a possible cause for dissemination of the GMO. Insects feeding on vaccinated chickens at a very specific timing when the vaccine strain multiplies in the wing web skin, can mechanically transmit the virus. Risk assessment showed that these events are not the cause for effective dissemination.

The overall level of risk to the environment of the use of Vectormune FP-LT is effectively zero.
It is to note that more than 4 billion doses of Vectormune FP-LT and Vectormune FP-LT+AE have been used in the USA and numerous other countries.

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

1. Recipient or parental organism characterization:

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

(select one only)

viroid	(.)
RNA virus	(.)
DNA virus	**(X)**
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	***Poxviridae family (Chordopoxvirinae subfamily)***
(ii)	genus	***Avipoxvirus***
(iii)	species	…
(iv)	subspecies	…
(v)	strain	***Cutter strain***
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	***FPV (=Fowlpox virus)***

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 parent strain is present in chicken flocks all over the world where vaccination is a practice.

Atlantic
Mediterranean
Boreal
Alpine
Continental
Macaronesian

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

(c) Is it frequently used in the country where the notification is made?

Yes **(X)** No (.)

(d) Is it frequently kept in the country where the notification is made?

Yes **(X)** No (.)
FPV vaccines are used in Hungary where fowlpox is present.

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

FPV cutter vaccine strain is known to replicate in chickens; it is also replicating in turkeys and other non-target birds (with very limited spreading capacities). Any replication in mammalian cells is considered "abortive".

Studies were conducted to compare the host range of the GMO to the FPV parent strain. First studies were conducted in turkeys, quails, doves, finches and in mammalian species -mice and pigs. The second studies were conducted in ducks, turkeys, quails, pigeons, guinea-fowls and pheasants. Results indicate that the high dose used for vaccinating non-target species triggered local signs of vaccine take in all species except for doves, finches, mice and pigs; it also showed that the host range was the same for both the GMO and the FPV parent strain.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
N/A

5. (a) Detection techniques
The virus can be grown in primary or secondary cultures of chicken cells such as embryonic fibroblasts, and causes a typical cytopathic effect (CPE). FPV can also be detected in wing web swelling a few days after vaccination by using the polymerase chain reaction (PCR on DNA extracted from the virus).

(b) Identification techniques
FPV virus can be identified by labeling viral foci with the aid of the immuno-fluorescence method using specific FPV antibodies. Alternatively, detection can be performed on DNA extracted from the virus using the polymerase chain reaction (PCR), using FPV genome specific primers.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (.) **No (X)***

If yes, specify
****: the parent FPV virus is a European and USDA licensed vaccine strain It is not listed in the Poxviridae section of Annex III of Directive 2000/54/EC of the European Parliament. It is non-pathogenic to humans, animals or plants.***

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) **No (X)** Not known (.)

If yes:

(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) of Directive 2001/18/EC

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

As viruses can only replicate in permissive cells derived from susceptible target species, the generation time for viruses can be defined as the average length of time from the release of the virion until it infects another cell. However from the practical point of view it is more useful to define it as the time elapsed from initial infection until the shedding of virions in the host. In birds, biosynthesis of FPV in dermal epithelium involves two distinct phases: a host response characterized by marked cellular hyperplasia during the first 72 hours and synthesis of infectious virus from 72 to 96 hours. Replication of fowlpox viral DNA in dermal epithelium begins between 12 and 24 hours post infection and is followed by the first appearance of infectious virus at 22 to 24 hours.

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

The same as in section 8.a)

(c) Way of reproduction: Sexual .. Asexual **(X)**

(d) Factors affecting reproduction:

Not applicable

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	

Fowlpox viruses do not form survival structures. The replication of avipoxviruses is restricted to avian species.

(b) relevant factors affecting survivability:

The replication of avipoxviruses is restricted to avian species. Fowlpox is a virus and viruses do not form survival structures. The parent strain and GMO as virus, are capable of replication only in live permissive cells. Laboratory tests determined the survivability of the parent strain in the litter to be of maximum of 8 hours. The parent strain and GMO were shown not to be transmitted from chicken to chicken.

10. (a) Ways of dissemination

The GMO and FPV parent strain replication in vaccinated chickens is limited and do not spread to non-vaccinated contact chickens. Dissemination of the vaccine into the environment will be restricted by its use in poultry houses. Vaccine spillage could occur at the farm during vaccine preparation (spillage by accident). Spilled quantities will be very limited, given the vaccine dose volume. The GMO was shown to have limited survivability in the environment if spilled. Dust bearing live spilled virus or insect feeding on vaccinated chickens where and when the virus replicates in the wing web skin are the two other ways of dissemination.

(b) Factors affecting dissemination

The parent strain multiplication in vaccinated birds is quite short; the vaccine does not spread from vaccinated birds. Survivability of the strain was found to be low, which decreases the chance of dissemination. In case of spillage, cleaning and disinfection procedures will even lower the risk of dissemination. The very limited place and time where and when the vaccine strain replicates in the skin greatly limits the possibility for an insect to effectively carry any significant quantities of live vaccine strain to another bird.

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),

None

C. Information relating to the genetic modification

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

Expression of the gB and UL-32 genes of LTV to act as antigens for immunization against Laryngotracheitis 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 (X) No (.)

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

(b) Identity of the vector
pNZ29R/LT-UL32gB

(c) Host range of the vector
E. coli

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

The LacZ gene was used to give a selectable phenotype to the plasmid. No antibiotic resistance gene was used.

antibiotic resistance (.)
other, specify ***LacZ***

Indication of which antibiotic resistance gene is inserted

Not applicable.

(e) Constituent fragments of the vector

The vector or homology plasmid consist of the followings: homologous non-essential region of the FPV genome; LTV gB-gene cDNA; LTV UL-32-gene cDNA, E. coli Lac Z-gene cDNA; synthetic promoter P17 directs transcription of the LacZ gene and a synthetic promoter Ps directs transcription of the LTV gB and LTV UL-32 genes.

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

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

Transfection of the plasmid into chicken embryo fibroblast (CEF) cell culture infected with FPV. The sequences of interest were recombined by homologous recombination into the FPV genome.

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

6. Composition of the insert

(a) Composition of the insert

The gene inserts were the LTV gB and UL-32 genes as well as the LacZ gene from E. coli.

(b) Source of each constituent part of the insert

The used genetic motifs are the synthetic promoters, Ps and P17, gB and UL-32 genes from laryngotracheitis virus as well as the LacZ gene from E. coli.

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

Immunization against laryngotracheitis virus and as a marker. The gB protein is an envelope glycoprotein and in herpesviruses mediates the virus entry, cell fusion and virus egress. In herpesviruses, the UL-32 protein plays a role in bringing pre-assembled capsids to the sites of virus DNA packaging. The LacZ gene encodes β-galactosidase, which functions as an excellent marker to distinguish the recombinant virus from parent FPV.

(d) Location of the insert in the host organism

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

Located in the FPV parent strain viral genome.

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

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

The organisms donating the genes and sequences are
(1) LT US field strain 632, obtained from Dr. Calvin L. Keeler, Jr., University of Delaware, donated the gB gene,
(2) a Japanese virulent strain of LT, NS175 was acquired from the Japanese Associates of Veterinary Biologics, which donated the UL-32 gene,
(3) E. coli donated the LacZ gene encoding β-galactosidase.

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (X)

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

2. Complete name

(i)	order and/or higher taxon (for animals):	**Herpesvirales**
(ii)	family name for plants	…
	family:	***Herpesviridae***
	subfamily:	***Alphaherpesvirinae***
(iii)	genus:	**Iltovirus**
(iv)	species:	***Gallid herpesvirus 1*** **(Infectious laryngotracheitis virus, ILTV)**
(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 (X) No (.) Not known (.)

If yes, specify the following:

(a) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.) **No (X)** 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 (X)***

If yes, specify

: *ILTV is not indicated in the EU Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. No other species than avian are known to be susceptible to ILTV infection and it is not considered as a zoonosis.

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) **No (X)** 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 ( ) **No (X)*** Not known (.)
Specify
*: ***the survival rate of the GMO is the same or decreased compared to the parent FPV.***

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

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

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) **No (X)*** Not known (.)
Specify
: All tests carried out on parent strain and GMO showed the same results: both strains are safe in the target species (chickens) and in non-target bird species; both strains do not disseminate from vaccinated chickens and turkeys, and have a very limited dissemination in other avian species; no replication was shown in mammalians)**. **Additional tested characteristics (tissue tropism, environment stability) of the GMO have remained the same than the ones of the parent strain.

2. Genetic stability of the genetically modified organism

The GMO was demonstrated to be genetically and phenotypically stable after five successive passages through chickens by the lack of reversion to virulence. The* in vivo *genetic stability of the GMO was confirmed using molecular tests to verify stability of the gene inserts and gene expression. Briefly, gene inserts were determined to be stable by Southern blot analysis and by DNA sequencing of important regions for gene inserts. Expression of the genes was verified using protein specific antisera to identify each gene product on plaque assays and western blotting.

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 virus can be grown in primary or secondary cultures of chicken cells such as embryonic fibroblasts, and causes a typical cytopathic effect (CPE). FPV can also be detected in wing web swelling a few days after vaccination by using the polymerase chain reaction (PCR) on DNA extracted from the virus.

(b) Techniques used to identify the GMO

GMO can be identified by labeling viral foci with the aid of the immuno-fluorescence method using specific antibodies against product expressed by the inserted ILT genes. Alternatively, detection can be performed on DNA extracted from the virus using the polymerase chain reaction (PCR), using probes specific to the inserted genes and insertion sites.

F. Information relating to the release

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

The purpose of the release is to carry out the necessary regulatory field trials in Hungary to complete the European registration dossier in accordance with Directive 2009/9/EC.

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):

Cooperating partner: Bábolna Tetra Kft.
Place of rearing pullet farm: Nagycenk, 9485, Kiscenki str 60, Hungary,
Place of laying farm 1: Vasegerszeg, 9661 Uraiújfalú, T.No.: 023/4
Place of laying farm 2: Zalavár 8392, Coordinate: 4973906, 1012222

(b) Size of the site (m^2): ***The vaccine will be used in 1 animal house of 1 farm (vaccination at the pullet farm; then pullet transferred to the 2 laying farms.***
(i) actual release site (m^2):
Pullet Farm: territory of 4250 (control) +2240 m^2 (Vectormune FP-LT+AE); Laying farm1.: territory of 4900 m^2. Laying farm2.: territory of 2560 m^2.

(ii) wider release site (m^2): not relevent

(c) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:
Next to the pullet farm is the "Nagycenki hársfasor" nature conservation area and the National Park Fertő-Hanság.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

Flora is not affected by FPV or the GMO.
On the farm only chickens are kept. The environment of the poultry house is closed, no exposure to any wild birds is expected.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Farm 1: A maximum of 30,000.pullets (30,000 doses) will be vaccinated

(b) Duration of the operation:
Vaccinating one flock takes a maximum of 5 days, if vaccination is performed in parallel in the same time.

(c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release

No special techniques are needed as the vaccine will not disseminate much during vaccination (limited vaccine spillage) and after (no shedding and dissemination expected).

It can be inactivated, easily eliminated, using common disinfectants (10% chlorine bleach, 10% iodine, or equivalent disinfectant). There are also procedures in place to dispose of the material and vaccine suspension remaining after vaccination.

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

The climate in the area is humid continental.

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.

Two vaccines containing the same recombinant strain are currently authorized and marketed in the USA, Argentina, Bangladesh, Bolivia, Brazil, China, Colombia, Costa

Rica, Ecuador, Egypt, Kuwait, Mexico, Pakistan, Peru, Philippines, Russia, Saudi Arabia, South Africa, Thailand and Ukraine.

The risk to public health is negligible for this vaccine. Human exposure will be limited to persons administering this vaccine or handling vaccinated chickens. Fowl poxvirus (FPV) is known not to be of public health significance and no known infection in humans has been reported.

More than 4 billion doses of the GMO have been used in the USA and in other countries and no adverse reactions or environmental impacts have been reported.

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

The interaction of the GMO with the environment is not different from the recipient organism FPV. The parent FPV virus strain has been safely used worldwide for more than 20 years in the poultry industry for the vaccination of chickens against Fowlpox disease; the GMO is registered since more than 10 years and has been used in many parts of the world safely.

1. Name of target organism (if applicable) ***Chicken***
   - (i) order and/or higher taxon (for animals): ***Galliformes***
   - (ii) family name for plants ***na***
   - (iii) genus ***Gallus***
   - (iv) species ***Gallus Gallus***
   - (v) subspecies ***G. Gallus Domesticus***
   - (vi) strain ***na***
   - (vii) cultivar/breeding line ***na***
   - (viii) pathovar ***na***
   - (ix) common name ***na***

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

   By vaccinating the chickens with the GMO, a protective immune-response is anticipated to develop against Laryngotracheitis disease and Fowlpox disease.

3. Any other potentially significant interactions with other organisms in the environment

   As the GMO does not spread from vaccinated chickens and diffusion in the environment through other means would be extremely limited, it is not expected to see any significant interactions with other organisms.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

   Yes (.) **No (X)** Not known (.)

   Give details

   Genetic stability results, as explained in paragraph A. 3.c., show that there is no such change expected in vaccinated birds. Passage studies in chicken have demonstrated the GMO does not become virulent upon passaging.
   Moreover, the vaccine is not likely to disseminate from vaccinated chickens and spread to non-vaccinated birds, further reducing the chances of these event occuring.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

The GMO was has a limited host range and in studies conducted by the applicant was only found to replicate in chickens and non-target birds species where a very significant quantity of the GMO was used for vaccination. Taking into account the lack of dissemination and spread of the GMO from the vaccinated chickens and the good vaccination and management practices applied, there are no risk of dissemination to non-vaccinated chickens, turkey or other bird (pheasant, guinea-fowls...) farms located in the vicinity of the vaccinated farms. Moreover, should spreading occur, the GMO is safe in these species, as demonstrated by the studies.

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

None

(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:

It is to note that replication of DNA virus (like the GMO) have much lower mutation rates and genetic variability than RNA viruses. Also virus 'superinfecting' a cell already infected by another virus is an unlikely phenomenon. However exchange of genetic material from the GMO to other virus in the chicken cells where it replicates, cannot be totally excluded in theory. This genetic recombination could then be remotely possible with other FPV viruses. The consequence may be the emergence of other FPV expressing the gB and UL-32 genes of LTV or part of these genes and at the same time GMO losing some of the inserted sequences. The potential for these events to occur would be no greater than for the parent strain FPV recombining with other FPV.
Overall, genetic exchange in vivo from the GMO to other organisms was not described.

(b) from other organisms to the GMO:

As explained above, genetic exchange in vivo from other FPV to the GMO is in theory possible but was never described. A very general phenomenon was reported in many vaccine strains present on the market, including parent strain of the GMO: integration of non-replicative portions of reticuloendotheliosis virus (another chicken virus) genome in fowlpox strains has been described. It was also checked that these insertions were totally inactive and did not compromise the safety of the vaccines containing it.

(c) likely consequences of gene transfer:

The chance of genetic exchange is not enhanced by the GMO and if this rare phenomenon occurs, the outcome is not expected to be harmful. Indeed the consequence may be the emergence of other FPV expressing the gB and UL-32 genes of LTV. This could induce immunisation and production of antibodies against LTV in the birds that would harbor the resulted virus. Also, it is to note that all inserted material that could be subject of this transfer is not leading to the expression of unexpected and/or undesirable traits.

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.):

Safety studies in non-target species and in the simulated environment, conducted by the applicant comparing the GMO to the FPV parent showed that the GMO was safe and no undesirable changes were seen when compared to the FPV parent strain.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

Not applicable, the GMO is not involved in the biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The vaccine strain can be detected by PCR as described above in section B.5.

Unless some unexpected event occurs, no specific monitoring of the GMO will occur as monitoring is not considered necessary.

2. Methods for monitoring ecosystem effects

The vaccinated chickens will be monitored regularly. Any adverse effects will be reported to the company and relevant authorities according to standard operating pharmacovigilance procedure.

It is to note that FPV is incapable of replication in humans or in mammals and was shown not to spread between chickens, thus there should be no virus spreading from the vaccinated flocks. The GMO is not able to survive more than 8 hours, not able to spread and is not pathogenic to animals or plants. No susceptible wild fauna should be present in the surroundings of the area, if non-target but susceptible birds are however present, they cannot receive by any means the dose necessary to induce a strain multiplication (no dissemination via vaccinated chickens, virus-bearing dust through spillage with only extremely low infecting dose as well as the highly improbable mechanical transmission through biting insects). No effect in the ecosystem is expected.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

In case this is necessary, the presence of the inserted sequence could be detected by PCR as described in section B.5.

4. Size of the monitoring area (m^2)

The monitoring area will be the farms where chickens will be vaccinated.

5. Duration of the monitoring

The monitoring on the farms will be done during the entire life of vaccinated birds.

6. Frequency of the monitoring

The general monitoring will be based on usual health visit in the farms.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Surfaces that will be used during vaccination will be cleaned using disinfectant as described in paragraph F.4(c). All materials used during vaccination will be disposed of using heat or disinfectant.

2. Post-release treatment of the GMOs

Apart from material used for the vaccination and possible limited spillage at time of preparation of the vaccine there is no expected release of the GMO in the environment.

3. (a) Type and amount of waste generated

During vaccination: Vaccine vials, needles, syringes and disposable aprons.

3. (b) Treatment of waste

Destruction according to procedures for infectious waste.

J. Information on emergency response plans

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

Possible dissemination of the GMO can occur through spilled vaccine. It can be either chemically destroyed by a 10% chlorine bleach, 10% iodine, or equivalent water-based disinfectant. Vaccine that may be spilled during the vaccination procedures will be cleaned using absorbent material and by prescribed disinfectants. All materials used during the cleaning procedures will be disinfected using the same products.
Taking into consideration the very limited amount of spillage possible, there is no need to plan a procedure for a general cleaning of the houses where the animals are kept to before the end of the bird production cycle. In any case, at the end of the cycle, poultry houses will be indeed disinfected using the usual disinfection and cleaning methods in force on the farm.

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

See paragraph J.1.

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

Not applicable. The GMO is not able to survive in the environment for longer than 8 hours. No spread to other animals is known.

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

The risk to public health is negligible for this vaccine. Human exposure will be limited to persons administering this vaccine or handling vaccinated chickens. Fowl poxvirus (FPV) is known not to be of public health significance and no known infection in humans has been reported.

Overall, the risk to the environment will be minimized by its use in a controlled environment in place at the farm. Any adverse drug reactions shall be reported to the company and authorities according to pharmacovigilance procedures in place.