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rHVT/ND-IBD (Project code: R051)

Live, genetically modified, frozen virus suspension for injection in broiler and layer pullets

**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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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:

rHVT ND IBDV (referred as R051) is a live genetically modified HVT vaccine strain containing ND and IBDV inserts. The vaccine developed for the prevention of virulent Marek's disease (MD (serotype-3 of MDV).), Infectious Bursal Disease (IBD) and Newcastle Disease (ND) in pullets. The active ingredient of the vaccine is a modified live turkey herpes virus (strain FC-126 of HVT), expressing the Fusion protein encoding (F) gene of Newcastle disease virus (NDV) and the capsid protein (VP2 gene) of Infectious Bursal Disease virus (IBDV).

In this document, information on the genetically modified vaccine strain (also referred to as R051) will be shared and discussed in accordance with Article 11 of Directive 2001/18/EC. A specific field trial request procedure will be launched with the Hungarian authorities (National Food Chain Safety Office, Directorate of Veterinary Medicinal Products). 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/18/02 |
| (c) | Date of acknowledgement of notification | 11/12/2017 |
| (d) | Title of the project | <i>Vaccination of chickens with a herpes virus of turkey vaccine with carrying VP2 gene of Infectious Bursal Disease Virus and F-gene of Newcastle Disease Virus diseases.</i> |
| (e) | Proposed period of release | <i>From April, 2018 - January 2019</i> |

2. Notifier

Name of institution or company:

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Budapest, Hungary

Tel: (+36 1) 262 95 05

Email: andras.pazsitka@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)

R051 vaccine contains the live, cell-associated and genetically modified turkey herpes virus (strain FC-126 of HVT), expressing the Fusion protein encoding (F) gene of Newcastle disease virus (NDV) and the capsid protein (VP2 gene) of Infectious Bursal Disease virus (IBDV).

The core construct of the R051 vaccine is the vaccine strain of Vectormune ND. This vaccine strain is a genetically modified form of the parent strain FC-126 of HVT containing one insert, the F-protein of Newcastle Disease Virus. This GMO was further modified by inserting the IBDV VP2 gene between the UL-44 and UL-45 gene of the HVT genome.

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

According to the studies performed by the applicant the GMO construct is stable (passages carried out in SPF birds and on CEF cells), the inserted sequence is well characterized and both of the Fusion protein (F gene) and the capsid protein (VP2 gene) are expressed after seven passages of the strain.

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 USA
- Notification number 1A89.RO SIF-RA

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 and on the environment is negligible in case of R051 vaccine strain. Human exposure will be limited to persons administering this vaccine or handling vaccinated chickens but even for them, the GMO is non-pathogenic. The parent strain of the active ingredient (of the GMO) is the HVT virus which is a naturally nonpathogenic virus. It is not listed in Annex III to Directive 2000/54/EC of the European Parliament.

The GMO is safe in the target species (chickens) and also safe in turkeys after spreading. The virus does not spread from vaccinated chickens to pheasants or pigeons. The genetic modification did not change the spreading or shedding properties of the R051 strain when compared to Vectormune ND or to the HVT strain. HVT strain cannot replicate in mammalian cells and the genetic modification did not change this property.

The overall level of risk to the environment of the use of R051 is effectively zero.

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) **Turkey herpes virus (HVT)**

other, specify ...

2. Name
- | | | |
|-------|---|--|
| (i) | order and/or higher taxon (for animals) | <i>Herpesviridae family</i> |
| (ii) | genus | <i>Mardivirus</i> |
| (iii) | species | <i>Meleagrid herpes virus 1</i> |
| (iv) | subspecies | ... |
| (v) | strain | <i>FC 126 strain</i> |
| (vi) | pathovar (biotype, ecotype, race, etc.) | ... |
| (vii) | common name | <i>HVT (=Turkey herpes virus)</i> |
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 including EC countries where vaccination is a practice.
- Atlantic
Mediterranean
Boreal
Alpine
Continental
Macaronesian
- (ii) No (.)
- (iii) Not known (.)
- (iv) Other (X)
- (c) Is it frequently used in the country where the notification is made?
- Yes (X)** No (.)
- Vaccines containing HVT are used in Hungary where virulent Marek's disease is present.***
- (d) Is it frequently kept in the country where the notification is made?
- Yes (X)** No (.)
- HVT vaccines are used in Hungary where virulent Marek's disease 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 | |

The HVT FC 126 strain is known to replicate in chickens; it is also replicating in turkeys and other non-target Galliformes birds (with very limited spreading capacities). Any replication in mammalian cells is considered "abortive".

Studies were conducted to compare the host range of the rHVT/ND-IBD with the HVT parent strain. Studies were conducted in turkeys, ducks, quails, guineafowls, pheasants, pigeons and in mammalian species. The safety of the R051 vaccine strain (rHVT-ND/IBD) on non-target species was assessed in turkeys, pigeons and pheasants as well. Results indicate that there were no changes in safety aspect of the HVT strain after the genetic modification, the strain remained apathogenic for each species.

- (b) If the organism is an animal: natural habitat or usual agroecosystem:
N/A
5. (a) Detection techniques
Immunostaining in culture on chicken embryo fibroblasts (black plaque assay) or by PCR.
- (b) Identification techniques
The parent strain can be identified by immunostaining method using HVT specific monoclonal antibodies. Alternatively, detection can be performed using polymerase chain reaction (PCR).
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
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
The recipient organism does not represent any hazard for domestic or wild animals.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Not applicable, the GMO is a virus, it is capable of replication only in live permissive cells. HVT is known to cause persistent infection in chickens and can be detected during the whole life of the animal. After inoculation of chickens the virus may spread to SPF turkeys after 35 days of contact exposure.

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

No information is available on the generation time.

(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 | |

Marek's disease viruses (MDV) do not form survival structures. The parent HVT and the rHVT/ND-IBD are both cell associated viruses, they lose infectivity when the cells that harbor them die. Survival in the environment is very short: it is 2 hours in wood shavings on 25°C (mimicking the environment of a poultry house).

Serotype 1 and 2 Marek disease viruses are able to replicate in the feather follicle epithelium of birds that are susceptible to the virus. MDVs shed by the feather dander may remain viable for a few months. However, the replication of HVT in the feather follicle epithelium is only limited and transient in chickens. No vertical or airborne infection with HVT is known. Between turkeys the spread happens probably by contact exposure.

(b) relevant factors affecting survivability:

Cell associated virus cannot survive in the environment after drying. In wet environment it loses infectivity within 2 hours.

10. (a) Ways of dissemination

HVT can spread from vaccinated to non-vaccinated chickens via dust from feather follicles. But even for naïve birds the vaccine is apathogenic and will not cause any clinical signs or macroscopic alterations.

- (b) Factors affecting dissemination

The parent virus, or the GMO is cell associated, the cell associated virus is not able to survive in the environment after the host cell dies. The dissemination is probably host dependent-

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),

Vectormune ND, Hungary.

Notification number: B/HU/12/01

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 F Fusion protein of Newcastle disease virus, and the structural capsid protein VP2 of Infectious Bursal Disease to act as antigens for immunization of chickens against Newcastle disease and Infectious Bursal 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
p45/46PecF
p44/45d46MCMVie1VP2SPA2

(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 an electable phenotype to the plasmid, but it is not present in the final construct. 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

Genes of interest, see B.5.

(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 HVT. The sequences of interest were recombined by homologous recombination into the HVT 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

The sequences of interest were recombined by homologous recombination into the HVT genome.

The vector or homology plasmid of the first genetic modification (insertion of the F-protein encoding gene into the HVT genome) consists of the followings: homologous non-essential region of the HVT genome; NDV F-gene cDNA; PEC promoter at the 5' terminus and polyadenylation signal at the 3' terminus. In case of the current modification IBDV VP2-gene cDNA; M CMV ie1 promoter at the 3' terminus and polyadenylation signal at the 5' terminus were introduced in the HVT genome.

6. Composition of the insert

(a) Composition of the insert

The previous (Vectormune ND) insert contains: NDV F-gene cDNA; PEC promoter at the 5' terminus and polyadenylation signal at the 3' terminus.

In case of the current modification (R051 vaccine): IBDV VP2-gene cDNA; M CMV ie1 promoter at the 3' terminus and polyadenylation signal at the 5' terminus were introduced in the HVT genome.

(b) Source of each constituent part of the insert

The used genetic motifs are the Pec- and M CMV IE promoter, and the SV40 poly A and synthesized short poli A addition sites.

The cDNA of the VP2 gene was obtained from the STC strain of IBDV by RT-PCR. Strain STC is a part of the classic IBDV strains.

The promoter (ie1) is the region of the Murine Cytomegalovirus (MCMV) immediate early (IE) promoter. The polyadenylation site is a synthetic polyadenylation site originated from SV40.

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

Expression of the inserted F (in a previous genetic modification) - and VP2 genes in the vaccinated animals for the active immunization against Newcastle disease and Infectious Bursal Disease. Active immunity against virulent Marek's disease will be elicited as well due to the antigenic relatedness of HVT virus to Marek's Disease Virus.

(d) Location of the insert in the host organism

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

Inserted into the HVT 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 inserted sequence (applied in present genetic modification) was derived from Infectious Bursal Disease virus STC strain by RT-PCR.

1. Indicate whether it is a:

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

2. Complete name

| | | |
|--------|--|----------------------------------|
| (i) | order and/or higher taxon (for animals): | ... |
| (ii) | family name for plants | ... |
| | family: | Birnaviridae |
| | subfamily: | ... |
| (iii) | genus: | Avibirnavirus |
| (iv) | species: | ... |
| (v) | subspecies | ... |
| (vi) | strain | STC |
| (vii) | cultivar/breeding line | ... |
| (viii) | pathovar | |
| (ix) | common name | Infectious Bursal Disease |

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
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 see at B 9. (b)
- (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 see at B 8. (a)
- (c) is the GMO in any way different from the recipient as far as dissemination is concerned?
 Yes (.) **No (X)** Not known (.)
 Specify ***Dissemination rate of the HVT strain was not affected by the genetic modification.***
- (d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
 Yes (.) **No (X)** Not known (.)
 Specify ***The pathogenicity of the rHVT/ND-IBD is not different from the parental HVT. It proposes no danger to chickens (target species), and to other non-target species.***
2. Genetic stability of the genetically modified organism
The expressed proteins of the F- and VP2 genes can be detected with immuno-staining method using specific monoclonal antibodies in the final product.
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)

Not applicable.

4. Description of identification and detection methods

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

The GMO may be detected using PCR .

- (b) Techniques used to identify the GMO

The identity of the GMO may be checked using PCR specific for the inserts or by specific immunostaining using monoclonal antibodies (black plaque assay).

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 field trials in Hungary to support 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: Broiler Farm:

Owner of the Broiler farm: Szimultán Baromfi-Coop Ltd.

Place of the broiler farm: 4565 Pusztadobos, topographical number 0128/9, Hungary; 4060, Balmaújváros-Nagyhát, topographical number. 0262/2

The vaccination will be done at the hatchery of Nagisz Baromfi-Coop Co. Ltd (4542 Petneháza, topographical number 095/9,Hungary. Derecske (4130 Derecske, Köztársaság u. 24.) by in ovo and subcutaneous application. Approximately 64000 broilers will be vaccinated.

After the vaccination the birds will be placed to Broiler Farm.

- (b) Size of the site (m²):

- (i) Actual release site (m²): ***in 54 equal animal houses (64000 broilers in each, in 2000 m²)***

animal houses:

animal house 1 and 5: approximately 1200 m²

animal house 2,3,4: approximately 1770 m²

- (ii) wider release site (m²): not relevant

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

Proximity to Csokaly fishing lake is 7 km. Proximity to Vaja-lake nature conservation area is 15 km Proximity to Látóképi fishing lake is 10 km. Proximity to Keleti water reservoir is 13 km. Proximity to the protected area of Hortobágy National Park is 37 km.

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

Flora is not affected by HVT 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:

A maximum of 64,000 pullets (64,000 doses) will be vaccinated.

- (b) Duration of the operation:

Subcutaneous or in ovo vaccination of birds takes a few hours.

- (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 to avoid spread. Standard business operations will be applied on the farm. The vaccine strain cannot spread to non-vaccinated chickens.

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

The climate in Hungary is humid continental. The weather is characterized by four well defined seasons, including at least one month with average temperatures below zero centigrade.

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.

The GMO is incapable of replication in humans or mammals .

The cell associated GMO is not able to survive, disseminate in other organisms than some Galliform birds and is not pathogenic to animals or plants.

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>Chicken</i> |
| (i) order and/or higher taxon (for animals): | <i>Galliformes</i> |
| (ii) family name for plants | <i>na</i> |
| (iii) genus | <i>Gallus</i> |
| (iv) species | <i>Gallus Gallus</i> |
| (v) subspecies | <i>G. Gallus Domesticus</i> |
| (vi) strain | <i>na</i> |
| (vii) cultivar/breeding line | <i>na</i> |
| (viii) pathovar | <i>na</i> |
| (ix) common name | <i>na</i> |

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Vaccination of the chickens with R051 strain will induce active immunity against Newcastle Disease, Infectious Bursal Disease and Marek's disease.

3. Any other potentially significant interactions with other organisms in the environment
As the GMO does not spread between 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. By keeping R051 vaccinated chickens together with contact control turkeys (the most susceptible species for HVT) the vaccine strain was able to spread to the turkeys but even for them the vaccine strain was safe.

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.

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 can replicate in turkeys, and some other Galliform birds. Spread is only known to turkeys, however no wild turkey population exists in the proximity of the release site. In clinical studies it has been shown that the virus is safe in turkey and in other wild birds that may harbor the virus.

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:

The chance of in vivo recombination between rHVT/ND-IBD and field NDV and IBDV strains and other viruses was considered. Since HVT replicates its DNA in the cell nucleus, recombination events with viruses replicating RNA (i.e. NDV and IBDV) in the cell cytoplasm are highly unlikely due to the different locations of genetic material and the different types of genetic material.

The chance of an in vivo recombination between rHVT/ND-IBD and field HVT or MDV viruses is theoretically possible. However, common vaccination practices in Europe and in the United States involve the mixture of HVT (serotype 3), SB1 (serotype 2), Rispens (serotype 1) and IBDV (serotype 1) vaccines. To date, no adverse events caused by the concurrent application of any of these vaccines or with field MDV (serotype 1) viruses have been reported. One case of experimentally produced recombination of MDV serotype 2 with MDV serotype 1 was reported but was not repeatable. The chance of an in vivo recombination event is unknown, but it is a remote possibility due to the similar replication cycle of HVT and other MDV serotypes. The potential for recombination of the rHVT/ND-IBD strain with virulent Marek's disease virus would be not greater than that may occur with current vaccines containing HVT.

The exchange of genetic material would be possible with other HVT, MDV strains, in case the same host cells become infected with more than 1 type of virus at the same time. There is evidence that suggests that, however infection of the same cells with different herpes viruses is possible, it is rare due to a phenomenon called superinfection inhibition (the prevention of infection of already infected cells by other viral particles of the same viral species). Due to superinfection inhibition there is only a very limited time (between 1-4 hours) for a cell to become infected with different herpes viruses. This phenomenon significantly reduces the chances of transfer of genetic material. If the GMO would lose both inserts (F gene and VP2 gene) due to the instability of the construct it would still result the naturally apathogenic FC-126 strains.

(b) from other organisms to the GMO:

Please see in G.7.a.

(c) likely consequences of gene transfer:

Please see in G.7.a

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.):

The safety studies were performed in the spirit of the European Pharmacopoeia Marek's Disease Vaccine (Live) monograph 0589 and no adverse reactions or safety concerns attributable to the vaccine strain have been reported. Additionally, the safety of the vaccine was also investigated in turkeys, pheasants and pigeons and also for them the vaccine strain is safe.

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.

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.

HVT is incapable of replication in humans or other mammals. The GMO is not able to survive, spread and is not pathogenic to animals or plants. No susceptible wild fauna is present in the surroundings of the area. No effect in the ecosystem is expected.

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

Not applicable since the transfer is highly unlikely.

4. Size of the monitoring area (m²)

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

5. Duration of the monitoring

Not applicable.

6. Frequency of the monitoring

Daily.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Surfaces that have been used during vaccination will be cleaned using the usual practice in the vaccination area after poultry vaccination. The vaccine strain does not survive after drying due to its cell associated nature. All materials used during vaccination will be put in special containers and destructed according to procedures for infectious waste. The animal houses will be cleaned and disinfected according to the procedure in force on the farm. This includes sweeping, cleaning with water, with detergent, rinsing and disinfection with formaldehyde gas twice. Any dead birds will be transported by Szatev Zrt. for decontamination. The litter from the poultry house where the animals were kept will be handled according to usual protocols on the farm, as no cell free virus is shed by the faeces and the cell associated virus is not able to survive in the litter or manure.

2. Post-release treatment of the GMOs

Used vaccine vials and other exposed materials will be transported back to the applicants laboratories. Pharmacy using closed, unbreakable leakage-free container and will be destroyed according to procedures for infectious waste. Unused vaccine will be returned to the sponsor by the study director.

3. (a) Type and amount of waste generated

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

4. (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

Vaccine that may be spilled during the vaccination procedures will be cleaned using absorbent material and by disinfectants usually used on the farm. All materials used during the cleaning procedures will be destructed according to procedures meant for the destruction of infectious waste. Poultry houses where the animals were kept will be 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

Not applicable. 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.

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.

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