EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Veterinary Medicines Division

## Committee for Medicinal Products for Veterinary Use

# CVMP assessment report for Nobivac Myxo-RHD Plus (EMEA/V/C/004989/0000)

Vaccine common name: Myxomatosis and rabbit haemorrhagic viral disease vaccine (live recombinant)

**Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted**

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# Introduction

The applicant Intervet International B.V. submitted on 4 September 2018 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Nobivac Myxo-RHD Plus, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 15 February 2018 as Nobivac Myxo-RHD Plus has been developed by recombinant DNA technology.

The approved indication is:

For active immunisation of rabbits from 5 weeks of age onwards to reduce mortality and clinical signs of myxomatosis and rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV1) and RHD type 2 virus (RHDV2).

Onset of immunity: 3 weeks.

Duration of immunity: 1 year.

The active substances of Nobivac Myxo-RHD Plus are two live recombinant myxoma-vectored RHD viruses (strain 009 and strain MK1899), expressing the capsid protein gene of classical or type 2 RHD viruses respectively, which trigger an active immune response against both myxoma and RHD viruses. The target species is rabbits. The product is intended for administration by the subcutaneous route.

Nobivac Myxo-RHD Plus is presented as a lyophilisate and solvent for suspension for injection containing live myxoma-vectored RHD virus strain 009 ($\geq 10^{3.0}$ and $\leq 10^{5.8}$ Focus Forming Units (FFU)) and live myxoma-vectored RHD virus strain MK1899 ($\geq 10^{3.0}$ and $\leq 10^{5.8}$ FFU) (lyophilisate fraction). The lyophilisate fraction is to be reconstituted before administration with the solvent provided.

Nobivac Myxo-RHD Plus is presented in packs:

- Plastic box containing 5 type I glass vials of lyophilisate (1 dose) and 5 type I glass vials of solvent (0.5 ml)
- Plastic box containing 25 type I glass vials of lyophilisate (1 dose) and 25 type I glass vials of solvent (0.5 ml)
- Cardboard box containing 10 type I glass vials of lyophilisate (50 doses) and cardboard box containing 10 type I glass vials of solvent (10 ml)

The rapporteur appointed is Esther Werner and the co-rapporteur is Consuelo Rubio Montejano.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC – full application.

On 12 September 2019, the CVMP adopted an opinion and CVMP assessment report.

On 19 November 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for Nobivac Myxo-RHD Plus.

## *Scientific advice*

Not applicable.

### *MUMS/limited market status*

The applicant requested eligibility of this application for MUMS/limited market by the CVMP, and the Committee confirmed that, where appropriate, the data requirements in the relevant CVMP guideline(s) on minor use minor species (MUMS) data requirements would be applied when assessing the application. MUMS/limited market status was granted as rabbits are considered a minor species.

# Part 1 - Administrative particulars

### *Detailed description of the pharmacovigilance system*

The applicant has provided documents that set out a detailed description of the pharmacovigilance system (DDPS), version dated in Annex 5.20. A statement signed by the applicant and the qualified person for pharmacovigilance (QPPV), indicating that the applicant has the services of a qualified person responsible for pharmacovigilance (PhV) and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country has been provided.

### *Manufacturing authorisations and inspection status*

A number of sites are involved in the manufacture, labelling and quality control (QC) of Nobivac Myxo-RHD Plus and the solvent.

Production of the active substance and the batch release of the finished product is performed by Intervet International B.V. in Boxmeer, Netherlands.

For all sites, appropriate and valid manufacturing authorisation and GMP certificates were presented. Specific inspections are currently not required.

### *Overall conclusions on administrative particulars*

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites were considered in line with legal requirements.

# Part 2 – Quality

### *Chemical, pharmaceutical and biological/microbiological information (quality)*

Nobivac Myxo-RHD Plus is a live, attenuated vaccine intended for active immunisation of rabbits against myxomatosis, classical RHDV1 and the variant RHDV2. The vaccine is mixed with the solvent "Solvent for Nobivac Myxo-RHD Plus" prior to subcutaneous injections into rabbits (throughout this document it is referred to as "Nobivac Solvent"). The vaccine does not contain any adjuvant or preservative.

## *Qualitative and quantitative particulars of the constituents*

## Qualitative and quantitative particulars

### *1.1 Composition*

**Composition per vial[1] of freeze-dried vaccine**

| Name of substance |
|---|
| Live myxoma-vectored RHD virus strain 009<br>Live myxoma-vectored RHD virus strain MK1899 |
| Hydrolysed gelatin<br>Pancreatic digest of casein<br>Sorbitol<br>Disodium phosphate dihydrate |

[1]Vials may contain 1 or 50 doses

**Composition per ml of solvent**

| Name of substance |
|---|
| Disodium phosphate dihydrate<br>Potassium dihydrogen phosphate<br>Water for injections |

The qualitative and quantitative particulars of the vaccine suspension and the solvent are described adequately. The necessary certificates are provided.

## *Container and closure*

The vaccine is presented lyophilised, enclosed in type I glass vials, closed with halogenobutyl rubber stoppers and aluminium capsules as presentations of 1 or 50 doses per vial, accompanied by the solvent Nobivac Solvent, which may be presented in glass (0.5 ml and 10 ml presentation) vials. The containers and the stoppers are described adequately. The necessary certificates are provided. The materials of the containers and stoppers are Ph. Eur. compliant.

## *Product development*

Nobivac Myxo-RHD Plus is the successor vaccine of the EU centrally licensed product Nobivac Myxo-RHD. It was developed to provide protection against the newly emerging variant of RHDV, RHDV type 2, against which the protection conferred by the already authorised vaccines was not sufficient.

Nobivac Myxo-RHD Plus contains not only one but two myxoma-vectored rabbit haemorrhagic disease strains: the classical strain 009 and variant strain MK1899. The myxoma virus is vectored with the respective VP60 protein of the RHDV strains; both strains are included in the vaccine and therefore protection against myxomatosis and both RHDV strains is conferred.

The maximum amount of virus in the product was reduced from $10^{6.1}$ FFU/dose to $10^{5.8}$ FFU/dose per strain, to not exceed the maximum amount of virus compared to the monovalent vaccine.

The extraneous agents test for live myxomatosis vaccines includes absence of antibodies against RHDV in live rabbits inoculated with the vaccine. As this is not possible in the case of Nobivac Myxo-RHD Plus, the applicant has a PCR test available which detects strains of wild-type RHDV in the final product but not the recombinant strains. The applicant deems it very unlikely that RHDV other than the recombinant strains would contaminate the vaccine, as RHDV does not grow in cell culture and master cell banks are free from the virus and no other ingredients of rabbit origin are used.

In all relevant parts of the dossier the data on the production and testing of strain MK1899 was added.

Virus titration which was performed by detection of a CPE (cytopathogenic effect) only is now also included in the immunofluorescence test (IFT) performed for identity.

The test on extraneous agents is performed in live rabbits or by PCR (validation of the PCR for wild type of RHDV to be presented yet).

Stability studies are ongoing.
A risk assessment on the photostability of the product stored in transparent secondary packaging was added.

For the single dose presentation, compared to Nobivac Myxo-RHD, the injection volume per rabbit was changed from 1 ml to 0.5 ml.

The description under product development was amended. Information on historical and scientific development was added.

## *Description of the manufacturing method*

The production process of the vaccine and the solvent is described in detail and relevant validation studies are provided. The production system is a RK-13 cell culture. Clarification on some minor issues during the production process was provided, including more detailed information on the batch size. Furthermore, some details in the validation reports were clarified.

The production procedure is performed in accordance with Good Manufacturing Practice and other relevant legislation. Only starting materials complying with the specifications in the monographs are used.

In brief, monolayers of RK-13 cells are infected with the myxoma-vectored RHDV strains; the virus is harvested, mixed with a stabiliser, filled into vials, lyophilised, stoppered and frozen at ≤ -15°C, or stored at 2-8°C.

The components of the solvent are mixed in a vessel, filled in glass vials and stoppered. Solvent is sterilised in the vials.

It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible and consistent manner. Respective validation reports are provided.

The in-process controls are adequate for this type of manufacturing process.

## *Production and control of starting materials*

## *Active substance*

Specifications of the active ingredients (Live myxoma-vectored RHDV strains 009 and MK1899) are defined and analytical methods are provided.

Stability studies are provided for the following intermediates of production:

Final bulk material may be kept at 2-8 °C for a maximum of 7 days before filling and lyophilisation. Satisfactory validation data is provided.

## *Excipients*

Specifications of excipients and other starting materials (e.g. materials of biological and non-biological origin, media) are defined and analytical methods are provided. Where applicable, the starting materials are in compliance with Ph. Eur. or other respective regulations.

## Starting materials listed in pharmacopoeias

Certificates of analysis have been provided and all conform to specifications in Ph. Eur.

## *Specific materials not listed in a pharmacopoeia*

## Starting materials of biological origin

All starting materials of animal origin which do not fall within the scope of Ph. Eur. 5.2.8 are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

Seed materials

The parent myxoma strain for the construct of strain 009 was derived from a rabbit vaccinated with an attenuated vaccine strain; the VP60 of RHDV1 was obtained from a diseased German rabbit in 1989.

The passage history of the construct is not described, it is stated that it was passaged in RK-13 cells for purification from non-recombinants.

For production of the master seed virus (MSV), virus was harvested from cell culture. Virus suspension was mixed with stabiliser, filled, freeze-dried and stored frozen. Information was provided on the volume of the aliquots of the MSV and the nature of the container.

Controls and tests carried out for sterility, exclusion of mycoplasma and extraneous agents on the MSV are described and the respective documents are provided. A risk assessment and justification is provided for several pathogens relevant for the source species, for which no specific test was performed. These justifications are considered satisfactory by the CVMP. Identity of the MSV for strain 009 was confirmed by PCR and sequencing. The respective report is provided. The complete quality control certificate for the MSV is also provided.

Working seed virus (WSV) is prepared as described in Part 2.B. and maximally 4 passages from the MSV. Final virus suspension is filled into portions, labelled and stored frozen. The complete quality control certificate for the WSV is provided.

A short summary on genetic engineering including description of the source materials is provided. A

report on the genetic stability after *in vitro* passage is provided.

The description and results of the production and control of the MSV and WSV for strain 009 and strain MK1899 are considered satisfactory.

The parent myxoma strain for the construct of strain MK1899 was derived from a rabbit vaccinated with an attenuated vaccine strain; the VP60 of RHDV2 was obtained from a diseased Spanish rabbit in 2012.

The passage history of the construct is described as passages in RK-13 cells for purification from non-recombinants.

For production of the MSV, virus was harvested from cell culture. Virus suspension was mixed with stabiliser, filled, freeze-dried and stored frozen. Information was provided on the volume of the aliquots of the MSV and the nature of the container.

Controls and tests carried out for sterility, exclusion of mycoplasma and extraneous agents on the MSV are described and the respective documents are provided. A risk assessment and justification is provided for several pathogens relevant for the source species, for which no specific test was performed. These justifications are considered satisfactory by the CVMP. Identity of the MSV for strain MK1899 was confirmed by PCR and sequencing. The respective report is provided. The complete quality control certificate for the MSV is provided.

WSV is prepared as described in Part 2.B. and maximally 4 passages from the MSV. Final virus suspension is filled into portions, labelled and stored frozen. The complete quality control certificate for the WSV is provided.

Concerning genetic stability of strain MK1899 the applicant refers to a study performed with Nobivac Myxo-RHD which contains only strain 009 and not strain MK1899. The omission of repetition of the study on genetic stability is accepted in this particular case, as the strains are highly similar, though not identical. But it can be considered that the biological properties of both vaccine strains are identical and they will have the same behaviour *in vitro* and *in vivo*.

Production system RK-13 cell culture

The passage history for the preparation of the actual MCS at Intervet is provided and the cells are in use for manufacture of vaccines since 1993, the description provided is considered sufficient. Information on the preparation of the MCS is provided.

Controls and tests carried out for sterility, exclusion of mycoplasma and extraneous agents on the MCS are described and the respective documents are provided.

A risk assessment and justification is provided for several pathogens relevant for the source species, for which no specific test was performed. These justifications are considered satisfactory by the CVMP.

The complete quality control protocol for the MCS is provided.

A test on tumourigenicity was not performed. A justification is provided, stating that the presence of any living cells in the product can be excluded due to the nature of the production process. Furthermore, the cell line is already used for manufacture of vaccines since 1993 and there are no indications that vaccination may lead to RK-13 derived tumours. Therefore the CVMP agrees with the conclusion that the testing is not necessary.

The MCS is considered as TSE compliant as the stock was already prepared in and is used since 1993.

It is stated that tests on identification and karyology were performed according to Ph. Eur. and it is referred to documents in which satisfactory information on isoenzyme analysis of RK-13 cells can be found confirming the species and genetic stability up to passage MCS+20; additional documents on karyology were provided.

The cell line RK-13 is considered suitable as production system for the viral antigens.

Starting materials from animal origin

The function, characteristics and origin of starting materials from animal origin is described. Satisfactory certificates of analysis for all substances used in the production of the vaccine are provided.

TSE risk assessment

An assessment was conducted in order to demonstrate that the risk of TSE transmission and propagation is minimised by the documented and recorded sourcing of animals (animal-derived material of known and controlled origin) and by the nature of the animal tissues used in manufacturing (low or no detectable infectivity).

A risk of transmission of animal spongiform encephalopathy agents of the vaccine Nobivac Myxo-RHD Plus is negligible. Furthermore, the vaccine is intended for use in rabbits which are not a TSE susceptible species.

The starting materials comply with the "Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" (EMEA/4010/01 incl. revisions).

Valid TSE certificates for all relevant suppliers of the respective substances are provided.

## Starting materials of non-biological origin

During production of the vaccine several media and buffers are used.
Used starting materials and preparation, as well as storage conditions for the listed solutions are described satisfactorily. Suppliers are listed as applicable or it is referred to the respective certificates of analysis.

## In-house preparation of media and solutions consisting of several components

Information regarding the qualitative and quantitative composition of all culture media and the stabiliser, their treatment processes and their storage conditions is provided in the dossier. All components are either tested for or treated to ensure that there are no contaminants or further assurance is given that there is no potential risk.

## *Control tests during the manufacturing process*

During manufacture the following in-process controls are carried out to ensure the quality parameters:

- Virus titration
- Sterility control
- Random test for filling of vaccine and solvent
  Amount of fill is regularly checked during the operation.

The in-process tests are deemed to be sufficient to control all critical steps in the manufacturing. The questions on the validation of the immunofluorescence test were satisfactorily addressed. A report on the missing controls and examples of immunofluorescence images were provided.

## *Control tests on the finished product*

Tests on the lyophilisate

- Identity and viral titre
  Validation of the test is provided and considered sufficient.
- Purity (sterility and mycoplasma)
  The tests on sterility and purity comprise freedom from bacteria and fungi and mycoplasmas according to Ph. Eur. 2.6.1 and 2.6.7 and monograph 0062. SOPs and validation reports are provided. The tests for sterility and absence of mycoplasma are considered as suitable.
  Furthermore, a test for specified extraneous agents in this case RHDV as required by Monograph 1943 slightly modified to fit the purpose is performed in rabbits. Additionally, the applicant proposes to introduce a PCR method to exclude wild type RHDV from the vaccine. The respective SOP is provided. However, as the validation of the method is still ongoing the validation report is not available and therefore an assessment of the method is not yet possible.
  The currently performed test in rabbits is considered as suitable for the purpose for now; it is appreciated that the applicant establishes a PCR method to replace the animal test.
- Residual humidity
  The test on residual humidity is based on volumetric Karl Fischer titration.
  The respective SOP is provided. The test is considered suitable.
- Appearance
  By visual inspection, in every batch produced, vials are inspected for capsule, volume, appearance and vacuum.
  The respective SOP is provided.

The description of the methods used for the control of the finished product and the respective specifications were provided.

Tests on the solvent

- Contents on average
  Test to ensure that the contents are within the limit set for the product.
- Appearance
  Visual inspection to ensure that the solvent is a clear, colourless liquid.
- Colour
  Check on the quality of product, by examination of the degree of coloration in accordance with Ph. Eur. 2.2.2.
- Clarity
  Check on the quality of the product by visual inspection according to Ph. Eur. 2.2.1.
- pH
  Test to demonstrate physiological acidity, via potentiometric method according to the Ph. Eur. 2.2.3.

- Identity
  Test to ensure that the vial is filled with the correct diluent. The solvent is tested for the presence of sodium, potassium and phosphate according to Ph. Eur. 2.3.1.
- Sterility
  Test to ensure freedom from bacteria and fungi, according to Ph. Eur. 2.6.1. and monograph 0062.

Validation data for all tests on lyophilisate and solvent are provided and are considered satisfactory.

## Batch-to-batch consistency

The production process results in a reproducible composition of the vaccine.

The applicant provides data from three independent production runs for antigen batches of strain 009 and strain MK1899, respectively. Antigen batches were tested for virus titre and sterility. All batches were demonstrated to be sterile. The virus titre was demonstrated as above the proposed minimum titre.

Three batches of vaccine and 3 batches of every solvent presentation were analysed and batch-to-batch consistency was demonstrated.

All control tests were performed as described in Part 2.E. The respective validation reports are provided in Part 2.B. The in-process and finished product tests are deemed to be sufficient to control all critical steps in the manufacturing process.

## *Stability*

Stability data were provided for: storage of antigen bulk before filling, the finished product, stability during transport, the in-use stability of the reconstituted product and the solvent.

Satisfactory validation data for the storage of antigen bulks for 7 days at 2-8 °C before filling and lyophilisation are provided.

The proposed shelf life for the final product with an internal storage of 24 months at -15 °C followed by an external storage of 24 months at 2-8 °C is supported. Stability testing is performed on 3 batches of finished product, even though the stability study is not yet finished; real-time stability data for the predecessor product Nobivac Myxo-RHD were also provided and are considered applicable also for Nobivac Myxo-RHD Plus due to their high similarity. Therefore, the full shelf life may already be granted before the real-time stability study for Nobivac Myxo-RHD Plus is finalised. However, the study will be finished for confirmation of results.

The in-use stability of 4 hours was sufficiently demonstrated.

The conditions for transport stability are proven for the storage at 30 °C for 3 days (accelerated stability). This is considered sufficient to demonstrate that the product is stable when transported at temperatures until 30 °C. Further data provided on the stability of storage at 42 °C for 3 days without any negative impact on the titre justify the information on storage as follows: "Store in a refrigerator (2 °C – 8 °C)".

A risk assessment on photostability of the lyophilisate packaged in transparent secondary packaging is provided and considered satisfactory.

A report and justification for the extension of the pH value of the 0.5 ml solvent presentation at the end of shelf life is provided and satisfactory.

The shelf life and storage conditions of the different solvent presentations are satisfactory for the 0.5 ml and 10 ml presentation.

## *Overall conclusions on quality*

Nobivac Myxo-RHD Plus is a recombinant live-attenuated vaccine intended for active immunisation of rabbits against myxomatosis, classical RHDV1 and the variant RHDV2. The vaccine lyophilisate is reconstituted with the solvent "Nobivac Solvent" prior to subcutaneous injections into rabbits.

The active substances are two attenuated strains of myxoma virus as vector backbone which express either the VP60 protein of the classical rabbit haemorrhagic disease virus (RHDV1) strain 009, or the variant rabbit haemorrhagic disease virus type 2 (RHDV2) strain MK1899. Both strains are included in the product. A stabiliser to protect the vaccine during freezing and storage is included.

No adjuvant or preservative is added. The minimum titre is $10^{3.0}$ FFU/dose and the maximum titre is $10^{5.8}$ FFU/dose for both strains contained in the product.

The composition of the solvent is the same as used for the solvent of Nobivac Myxo-RHD and Intervet's series of live injectable vaccines for cats and dogs (Nobivac Solvent).

The qualitative and quantitative particulars of the vaccine suspension, the solvent and the containers are described adequately. The necessary certificates are provided.

The section on development of the product was revised. Nobivac Myxo-RHD Plus is an update of the predecessor product Nobivac Myxo-RHD.

The production process of vaccine and solvent is described in detail and relevant validation studies are provided. The production system is a RK-13 cell culture. Clarification on some minor issues during the production process was provided, including more detailed information on the batch size. Furthermore, some details in the validation reports were clarified.

All starting materials comply with the respective provisions of Ph. Eur. and the TSE risk assessment is adequate. The respective certificates of suitability are provided. In general the description and results of the production and control of the MSV and WSV for strain 009 and strain MK1899 are considered satisfactory.

The relevance of a study on genetic stability for strain MK1899 was reasoned by the applicant and considered satisfactory. Information on the karyology of the cell line was provided. Regarding the RHDV strains 009 and MK1899, data of isolations and records of the origin were provided.

The in-process and finished product controls performed ensure a consistent production of Nobivac Myxo-RHD Plus. Data on critical controls on the immunofluorescence test performed for viral titration and identity test were provided; the test method is considered valid. A finished product control on the appearance of the lyophilisate was added to the list of final product controls.

The production process results in a reproducible composition of the vaccine. Three batches of vaccine and 3 batches of every solvent presentation were analysed and batch-to-batch consistency was demonstrated.

Data were provided for stability during storage of antigen bulk before filling, stability of the finished product, stability during transport, the in-use stability of the reconstituted product and the solvent.

The proposed shelf life for the final product with an internal storage of 24 months below -15 °C followed by an external storage of 24 months at 2-8 °C was proposed. Stability studies are on-going and, due to the similarity with Nobivac Myxo-RHD, the same shelf life is accepted. The applicant

states that any possible out-of-specification results will be appropriately handled. The in-use stability of 4 hours is also supported.

The product may be transported at temperatures up to 42 °C up to three days, as accelerated stability data were provided. The statement "Store refrigerated (2 ºC – 8 ºC)" is therefore accepted. The shelf life and storage conditions of the remaining solvent presentations of 0.5 ml and 10 ml are acceptable.

In conclusion, the production and quality of Nobivac Myxo-RHD Plus are adequately described and controlled and comply with the respective legal requirements including the TSE risk assessment.

# Part 3 – Safety

## *Introduction and general requirements*

Nobivac Myxo-RHD Plus is a vector vaccine which contains the live myxoma-vectored rabbit haemorrhagic disease (RHD) virus strain 009 and the live myxoma-vectored RHD virus strain MK1899. Both myxoma vaccine strains express the capsid protein gene of RHD: strain 009 the classical RHD virus type 1 and strain MK1899 the type 2. The myxoma vector virus MK1899 is derived from strain 009. No adjuvant or preservative is included.

The vaccine is intended for active immunisation of rabbits from 5 weeks of age onwards, including pregnant does, against myxoma virus, RHDV1 and RHDV2 by subcutaneous (SC) use. Onset of immunity is proposed with 3 weeks and duration of immunity with one year after one vaccination.

The vaccine is presented in freeze-dried form with accompanying solvent. The maximum dose of each vaccine strain is $10^{5.8}$ FFU/dose, resulting in a maximum amount of $10^{6.1}$ FFU/dose for both strains together. The volume of one dose varies between 0.5 ml (single dose presentation) and 0.2 ml (multi-dose presentation).

As the vaccine contains two genetically modified organisms (GMOs) according to Directive 2001/18/EC as amended, it completely falls under the scope of this Directive. Usually, a full set of data should be provided but as data had been gained with a similar GMO construct already authorised in Nobivac Myxo-RHD, it is acceptable to fulfil only parts of the requirements with Nobivac Myxo-RHD Plus according to guideline EMEA/CVMP/004/04. As rabbits are a minor species, the guideline EMA/CVMP/IWP/123243/2006-Rev.3 is also applicable. All laboratory studies have been carried out in accordance with GLP using batches with maximum antigen content. The field trials have been conducted according to the principles of good clinical practice (GCP).

## *Safety documentation*

Five laboratory safety studies were conducted to investigate the safety of Nobivac Myxo-RHD Plus and included: two laboratory studies (one in commercial hybrid rabbits and one in pet rabbits) investigating the safety of the administration of a ten-fold overdose, repeated dose and spread to unvaccinated rabbits. One study addresses the reproductive performance and two further studies a possible reversion to virulence of strain MK1899. All the other 12 safety studies were performed with the already authorised precursor product Nobivac Myxo-RHD containing only vaccine strain 009. In addition, safety and relevant performance measures were investigated in five field studies also performed with Nobivac Myxo-RHD.

The vaccine was administered by the subcutaneous route, as recommended. Laboratory studies were reported to be GLP compliant and carried out in rabbits of the minimum age recommended for

vaccination, using production batch containing $10^{6.1}$ FFU/vial of strain 009 and $10^{6.0}$ FFU/vial of strain MK 1899.

Further studies applicable to live vaccines and GMO products were conducted in the predecessor product Nobivac Myxo-RHD to investigate the dissemination of a single dose of the vaccine strain, the spread to non-target species and reversion to virulence of vaccine strain 009.

In general, all studies were carried out in compliance with Ph. Eur. monographs 0062, 5.2.6, 1943 and 2325.

| ***Study title*** |
|---|
| Safety of the administration of an overdose followed by one dose and evaluation of spread |
| Safety of the administration of an overdose followed by one dose and evaluation of spread |
| Safety of the subcutaneous administration of one dose in pregnant rabbits |
| Passaging of myxoma vectored RHDV virus strain MK1899 in SPF rabbits (genetic stability) |
| Determination of increase in virulence myxoma vectored RHD virus strain MK1899 subcutaneously administered to SPF rabbits |
| Safety of the administration of an overdose followed by one dose of Nobivac Myxo-RHD vaccine virus in dwarf rabbits |
| Study to determine the potential for spread of via the blood of vaccinated animals |
| Study to investigate the safety of myxoma-RHDV in mice |
| Study to investigate the safety of myxoma-RHDV in chickens |
| Study to determine the safety of an administration of myxoma-RHDV to European brown hares |
| Safety of the administration of an overdose followed by one dose and evaluation of spread of myxoma/RHDV vaccine virus in rabbits. |
| Determination of increase in virulence and dissemination of recombinant myxoma/RHD vaccine virus |
| Study to determine the dissemination in vaccinated rabbits |
| Study to investigate the effect of Nobivac Myxo-RHD in dwarf rabbits co-infected with myxoma virus |
| Administration by non-prescribed routes |
| Nucleotide sequence determination of myxoma/RHDV master-seed and 6th passage virus |
| Study to investigate the safety of co-administration of myxoma-RHDV and commercially available myxoma vaccine (Dervaximyxo SG33) |

Explanation: The first five studies at the top of the table are performed with Nobivac Myxo-RHD Plus. The other studies were conducted with the precursor product Nobivac Myxo-RHD and were provided during the marketing authorisation procedure of this product or as post-authorisation studies following PSUR assessment.

## *Laboratory tests*

In every study report concerning Nobivac Myxo-RHD Plus a GLP Compliance Statement, a Statement of Reliability and a Quality Assurance Statement is provided. All of these statements are signed and dated.

## *Safety of the administration of one dose*

No special laboratory study was performed to evaluate the safety of a single maximum dose of Nobivac Myxo-RHD Plus in rabbits, but the applicant conducted three combined overdose and repeated dose studies, which represents the worst-case scenario.

## *Safety of one administration of an overdose*

One pivotal study and two supportive overdose laboratory studies were provided (one in SPF commercial hybrid rabbits, one in SPF Dutch belted rabbits and one in non-SPF dwarf rabbits, respectively).

The safety of a tenfold overdose ($10^{6.9}$ FFU/dose of each strain) administered by SC injection to 5-week-old SPF New Zealand White (NZW) rabbits was examined in this study. The following groups were assigned; the control animals were housed in the same cages as the vaccinated rabbits:

- Group 1: 11 rabbits were vaccinated with a tenfold overdose of Nobivac Myxo-RHD Plus dissolved in 2 ml Nobivac Solvent
- Group 2: 11 rabbits were inoculated with Nobivac Solvent (2 ml)

Three weeks after the first vaccination a repeated single dose ($10^{5.9}$ FFU/dose of Myxo-RHDV1 and $10^{5.8}$ FFU/dose of Myxo-RHDV2) was applied to the same animals via SC injection:

- Group 1: 11 rabbits were vaccinated with a maximum single dose of Nobivac Myxo-RHD Plus dissolved in 0.5 ml Nobivac Solvent
- Group 2: 11 rabbits were inoculated with Nobivac Solvent (0.5 ml)

This study meets the requirements of Ph. Eur. Monograph 1943. No clinical signs were noted during the observation period of five weeks. After the administration of a tenfold overdose a majority of the vaccinated rabbits showed slight swellings of the injection sites (0.5 cm in diameter for a maximum of 4 days) as well as a slight swelling of the local lymph nodes. The reactions were noted in the first 14 days after vaccination and disappeared during the following 7 days. After administration of a repeated dose similar reactions were noted in some animals. No significant increase of body temperatures compared with controls was observed. At the beginning of the study all animals were negative for specific antibodies. In addition, it was recorded that the virus did not spread to the control group because they remained seronegative to myxoma virus, RHDV1 and RHDV2 and healthy.

The second study, performed in SPF Dutch Belted rabbits, was designed identically. No clinical signs were observed and similar findings concerning the injection sites were noted. The body temperature was significantly slightly higher in vaccinated animals compared to the controls and no spread was detected, too.

A third study was performed with Nobivac Myxo-RHD in dwarf rabbits to evaluate a tenfold overdose followed by a repeated dose. Thirty non-SPF myxoma-antibody-negative Netherlands dwarf rabbits of six weeks of age were distributed to the following groups:

- Group 1: 17 rabbits received Nobivac Myxo-RHD reconstituted in 1 ml solvent SC in the scruff of the neck:
  1st vaccination – titre of $10^{7.1}$ FFU/dose (10x overdose)
  2nd vaccination – titre of $10^{6.0}$ FFU/dose 21 days later (1x repeated dose)
- Group 2: 7 rabbits 1 ml Nobivac Solvent SC in the scruff of the neck, two inoculations 21 days apart
- Group 3: 6 rabbits remained as untreated controls

During clinical observations, clinical signs untypical for myxomatosis were noted in groups 1 and 2

but no myxomatosis specific signs were observed. Only a slight increase in body temperature was noted after the first vaccination. All rabbits were seronegative against myxoma virus at the start of the study and group 1 developed specific antibodies as expected. However, all rabbits had specific antibodies against RHDV from the start of the study. It was concluded that non-pathogenic caliciviruses were circulating at the rabbits' supplier farm, as the parent rabbits were also positive. In addition, administration of Nobivac Myxo-RHD was regarded as safe for dwarf rabbits.

It can be concluded, that Nobivac Myxo-RHD Plus is considered as safe for the target species including pet rabbits.

## *Safety of the repeated administration of one dose*

This topic is covered by the overdose studies where a repeated dose was given three weeks after the initial overdose.

## *Examination of reproductive performance*

The safety of the reproductive performance was investigated in accordance with Ph. Eur. monograph 1943 in the study, where one maximum dose ($10^{5.9}$ FFU/dose of Myxo-RHDV1 and $10^{5.8}$ FFU/dose of Myxo-RHDV2) was administered by SC injection to pregnant nulliparous does 7 days or 21 days after mating. The following groups were assigned:

- Group 1: 10 pregnant does were vaccinated 7 days after mating with a maximum single dose of Nobivac Myxo-RHD Plus reconstituted in 0.5 ml Nobivac Solvent
- Group 2: 10 pregnant does were vaccinated 21 days after mating with a maximum single dose of Nobivac Myxo-RHD Plus reconstituted in 0.5 ml Nobivac Solvent
- Group 3: 10 pregnant does were inoculated 7 days after mating with 0.5 ml Nobivac Solvent
- Group 4: 10 pregnant does were inoculated 21 days after mating with 0.5 ml Nobivac Solvent

No vaccine-related clinical signs were noted during the study and no differences of body temperatures were detected between the groups. The kindling results were normal for does of the first breeding season. No differences could be observed between vaccinated groups and control groups. Does and offspring appeared normal and healthy. Two kittens died during delivery and three does were found not pregnant (one doe became sick [control group], and two does probably did not conceive as during post mortems no abnormalities were found). However, this study is considered as supportive to demonstrate the reproductive safety in the target species as the conception rate of the mated does was satisfactory and in the provided pathological reports of barren does and dead kitten no indication of a negative influence of the vaccination could be detected.

Studies in breeding bucks have not been performed, as well as any examinations during lactation. No special warning in the SPC concerning lactation is considered necessary in line with other products.

## *Examination of immunological functions*

The applicant stated that the vaccine does not adversely affect the immunological functions as two main virulence factors are deleted from the myxoma vector genome. Myxoma virus may act potentially immunosuppressive but no study has been performed on the subject of this aspect. However, during the safety studies no adverse effects in this regard were observed. It is therefore unlikely that this vaccine will have an adverse effect on immunological functions.

## *Special requirements for live vaccines*

## Spread of the vaccine strain

Unvaccinated control groups were housed together, in direct contact with the vaccinates in one cage during the combined overdose/repeated dose studies. The controls did not seroconvert to myxoma virus or RHDV1 or RHDV2 and no clinical signs were noted. No examination of shedding was performed but in the studies conducted with the predecessor product no indication of shedding was found.

Additional studies performed for the marketing authorisation procedure of Nobivac Myxo-RHD are provided:

For examination of a potential transmission of the vaccine strain 009 by biting insects from vaccinated rabbits to naïve animals study was performed in 8-week-old SPF NZW rabbits vaccinated with a high dose of Nobivac Myxo-RHD.

- Group 1: 6 rabbits were vaccinated SC with $10^{6.8}$ FFU/dose of Nobivac Myxo-RHD

Several blood samples were collected at different time points and these samples were partly spiked with strain 009 as positive control. No virus re-isolation was possible from the non-spiked samples, but it was possible from all spiked samples. As for infection of a naïve animal a minimum virus titre needs to be transferred, spread of strain 009 via biting insects was regarded as not possible.

In addition, studies have been performed in mice, chickens and European brown hares, for which myxomatosis infection is a known potential risk as well. No signs of myxomatosis were detected within the vaccination groups of mice, one-day-old chickens and hares. No seroconversion or any signs of myxomatosis infection could be detected in the co-housed control group of the same species. For further support the combined overdose/repeated dose study performed with Nobivac Myxo-RHD is also provided. No spread of vaccine strain 009 was observed to unvaccinated control rabbits in contact with vaccinated animals.

As both strains are very similar CVMP considers the results of the studies conducted with Nobivac Myxo-RHD to be transferable to strain MK1899. In conclusion, it is accepted that the vaccine strains included in Nobivac Myxo-RHD Plus do not spread to unvaccinated rabbits or other animals.

## Dissemination in the vaccinated animal

No study addressing dissemination was performed with Nobivac Myxo-RHD Plus. However, dissemination of vaccine strain 009 in vaccinated rabbits was investigated with Nobivac Myxo-RHD in four studies.

In this study, 5-week-old SPF NZW rabbits were vaccinated with one maximum dose by SC injection. After five days the rabbits were sacrificed and samples were collected. The vaccine strain was re-isolated from the skin of the injection sites and the local lymph nodes. All other examined tissues (liver, kidney, spleen, genital organs, tonsils, nasal mucosae, blood, eyelids, lungs, and large intestine) were found negative. Nasal and conjunctival swabs were also tested negative.

In the second similar study, virus re-isolation was also successful from the skin of the injection sites and the draining lymph nodes, but not from other tissues (liver, kidney, lungs, genital organs, nasal mucosae, eyelids, anal/rectal skin, spleen and tonsils) or from nasal and conjunctival swabs, whereas virus isolation was possible in each case from spiked samples of the same tissues. Virus isolation from lymphocytes as carrier cells of the virus through the rabbit body after infection was also not successful.

In another study**,** 5-week-old dwarf rabbits were vaccinated and challenged with field myxoma virus to examine if it is possible to re-isolate vaccine virus in conjunctival swabs of rabbits challenged with a wild-type myxoma virus before or after vaccination with Nobivac Myxo-RHD. The reason for this was reporting of adverse events in PSURs. The following groups were included in this study:

- Group 1: 4 rabbits were vaccinated with Nobivac Myxo-RHD and challenged 21 days post vaccination.
- Group 2: 3 rabbits were vaccinated with Nobivac Myxo-RHD and challenged 7 days post vaccination.
- Group 3: 4 rabbits were vaccinated with Nobivac Myxo-RHD and challenged 3 days post vaccination.
- Group 4: 4 rabbits were vaccinated with Nobivac Myxo-RHD and challenged on the same day.
- Group 5: 4 rabbits were inoculated with virulent myxoma virus and vaccinated with Nobivac Myxo-RHD 3 days later.
- Group 6: 4 non-vaccinated control rabbits were challenged.

During the first three days post challenge no clinical symptoms were noted. The clinical signs noted subsequently could not be assessed as it was not clear if there was an efficacy problem or if the challenge strain was not correctly administered. In Group 1, only one out of four animals was completely protected when challenged at the time of onset of immunity. In Group 2, two rabbits out of three and in Group 3 one rabbit out of four remained healthy, whereas all other animals in these three groups developed myxomatosis-like symptoms. However, demonstration of onset of immunity was not intended here and all animals in groups 4 to 6 had to be euthanised 11-12 days after challenge, indicating a missing protection against the challenge. During the post authorisation safety study in pet rabbits the vaccine was evaluated to a comparator product and no differences between the groups were noted (see field studies).

Serology results indicated an immune response in all animals depending on the time point of vaccination.

No virus isolation was possible before challenge from conjunctival or rectal swabs. After challenge, vaccine virus 009 was found in conjunctival swabs of nearly all groups.

However, dissemination of vaccine strain 009 to eyes was demonstrated when vaccinated dwarf rabbits were infected with wild-type myxoma virus at the time of or shortly after vaccination with Nobivac Myxo-RHD. Therefore, the vaccine strain is able to disseminate to the eyes of vaccinated animals if challenged.

The last dissemination study, also a post-authorisation study, was performed to examine whether vaccine strain 009 is able to infect naïve SPF NZW rabbits via ocular or nasal route. Two groups were assigned with two rabbits each per vaccine dilution:

- Group E: Subgroups E0 – E4 were inoculated with solvent or Nobivac Myxo-RHD ($10^{1.2}$ FFU or $10^{2.2}$ FFU or $10^{3.2}$ FFU or $10^{4.2}$ FFU), via eye drop
- Group N: Subgroups N0 – N4 were inoculated with solvent or Nobivac Myxo-RHD ($10^{1.2}$ FFU or $10^{2.2}$ FFU or $10^{3.2}$ FFU or $10^{4.2}$ FFU), via nose drop

No clinical signs, no serological response and no shedding (nasal/conjunctival) could be observed, indicating that no immune response or infection occurs after ocular/nasal inoculation of vaccine strain 009.

As both strains are very similar, CVMP considers the results of the studies conducted with Nobivac Myxo-RHD to be transferable to strain MK1899. In conclusion, it is accepted that the vaccine strains included in Nobivac Myxo-RHD Plus do only in exceptional cases disseminate to the eyes of

vaccinated rabbits. If so, no infection will be triggered in contact animals.

## Reversion to virulence of attenuated vaccines

The reversion to virulence of each vaccine strain was investigated in four studies, in accordance with the requirements of Ph. Eur. 5.2.6 and Ph. Eur. 1943 monographs. The two studies performed for the marketing authorisation procedure of Nobivac Myxo-RHD are provided for vaccine strain 009 and two new studies were performed with vaccine strain MK1899.

In this study**,** 5-weeks-old SPF NZW rabbits were vaccinated with one maximum dose of Nobivac Myxo-RHD ($10^{6.1}$ FFU/dose). The vaccine virus was re-isolated from samples of skin and lymph nodes, homogenised and administered to the next group. This procedure was repeated up to the 6$^{th}$ passage. A comparative part of this study was performed in the following groups:

- Group 1: 5 rabbits received 2x 0.1 ml of Nobivac Myxo-RHD intradermally ($10^{3.0}$ FFU/0.1 ml).
- Group 2: 5 rabbits received 2x 0.1 ml of the homogenate from passage 6 intradermally ($10^{3.0}$ FFU/0.1 ml).
- Group 3: 5 rabbits served as untreated control.

No clinical signs and no significant differences concerning body temperatures were observed during this study, indicating no increase in virulence.

In another study**,** the MSV and the back passage virus 6 of vaccine strain 009 were compared by sequence analysis. The results confirmed that six animal passages did not lead to any instability of the insert or the myxoma region surrounding the RHDV1 insert.

Also another study was performed with the MSV+1 of vaccine strain MK1899. The vaccine strain was administered to 7-week-old SPF NZW rabbits with a dose of $10^{5.3}$ FFU/dose. Five animal passages were conducted. No indication of reversion to virulence was noted. A sequence analysis of the MSV+1 and the 5$^{th}$ passage was also carried out and four point mutations were found, probably due to tissue sample-related contaminations in the pooled tissue sample.

The aim of this study was to compare the MSV+1 of vaccine strain MK1899 with its 5$^{th}$ passage to evaluate possible reversion to virulence.

- Group 1: 10 rabbits received MK1899 MSV+1 with $10^{4.3}$ FFU/dose per SC injection
- Group 2: 10 rabbits received MK1899 passage 5 with $10^{4.3}$ FFU/dose

No clinical signs were noted and no significant differences in body temperature were observed. Therefore, no reversion to virulence could be noted.

In summary, it is concluded that no reversion to virulence was observed following six or five passages *in vivo* for both virus strains included in this product.

## Biological properties of the vaccine strain

For this part of the dossier only a short description addressing this point (the same as in the dossier of Nobivac Myxo-RHD) has been provided. This short summary is acceptable as a more detailed description of the properties and their assessment is included under environmental risk assessment.

## Recombination or genomic reassortment of the strains

No study was performed with Nobivac Myxo-RHD Plus to evaluate this topic.

In this study Nobivac Myxo-RHD was mixed with a commercial dose of a live attenuated licensed myxomatosis vaccine.

- Group 1 (5 rabbits): single dose (0.1 ml) of Dervaximyxo SG33.
- Group 2 (4 rabbits): unvaccinated controls, housed together with Group 1.
- Group 3 (5 rabbits): 0.1 ml mixed from 0.05 ml Nobivac Myxo-RHD and 0.05 ml Dervaximyxo SG33.
- Group 4 (4 rabbits): unvaccinated controls, housed together with Group 3.

The co-administration of the recombinant myxomatosis vaccine did not result in any clinical signs typical for myxomatosis or any other adverse effects.

The risk of recombination is considered as more eminent if latently infected animals would be vaccinated or if vaccinated animals would be infected before the onset of immunity. Parallel infection of the same cell with field myxoma virus and a vaccine strain may result in a re-activation of the original myxoma strain used for the GMO if the MGF and M11L genes may be transferred. Until now no reports have been received that suggest a recombination of strain 009 and Shope fibroma virus. The risk of reassortment was evaluated in detail by the applicant under environmental risk assessment and is considered as very low.

### *User safety*

A user safety assessment in line with the guideline on user safety for immunological veterinary medicinal products (EMEA/CVMP/543/03-Rev.1) was not provided. However, as the main route of potential exposure is accidental self-injection and myxomatosis and RHD are not considered as zoonotic diseases, the product is not expected to pose any risk to the person handling the product or the person who is in contact with vaccinated animals. No adjuvant and preservative are present in the vaccine.

The CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC

### *Study of residues*

No studies on residues have been performed.

## MRLs

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009. Consequently, it is considered that there is no need to perform residue studies for Nobivac Myxo-RHD Plus and a withdrawal period of zero days is accepted.

## Withdrawal period

The withdrawal period is set at zero days.

### *Interactions*

The applicant has not provided data investigating interactions of the vaccine with other veterinary immunological products and therefore proposes to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary

medicinal product therefore needs to be made on a case-by-case basis.'

## *Field studies*

No field studies performed with Nobivac Myxo-RHD Plus are provided. According to EMA/CVMP/IWP/123243/2006-Rev-3 (MUMS/Limited market guideline), it is acceptable for immunological veterinary medicinal products containing a GMO to provide data generated for a similar (precursor) construct already authorised. Furthermore, in this guideline is stated that if laboratory studies adequately demonstrate the absence of a significant target animal safety risk, field studies are not required. In addition, in the guideline EMEA/CVMP/004/04 (Guideline on live recombinant vector vaccines) is stated that data from other assessments performed with the same vector but other inserted sequences can be used as long as the new insert does not change the characteristics and specifications of the final construct.

Four field studies and a post-authorisation safety field study (PASS) are provided, which were performed with Nobivac Myxo-RHD. Two trials in pregnant does and two trials with 5-week-old rabbits (offspring from the studies with pregnant does) were performed. All animals received one commercial dose of vaccine by SC injection.

Slight body temperature increases and soft swellings at the injection sites, which disappeared completely within a few days, were observed in the vaccinated groups. The regional lymph nodes were not examined after the vaccination.

Regarding the reproductive performance the results were comparable between vaccination and control groups and in line with expected breeding results of NZW rabbits. Reproductive safety was investigated in does vaccinated on the 7$^{th}$ day or the 21$^{st}$ day of pregnancy. The product was found to be safe when used in pregnant animals.

A PASS was performed in 461 pet rabbits including 252 dwarf rabbits of different ages. One group was vaccinated with Nobivac Myxo-RHD and the other group with a licensed vaccine against myxomatosis and RHDV1. The clinical signs observed were comparable in both groups, except for the residual pathogenicity, which was observed only in the control group. Several adverse events were reported, but also comparable in both groups. Based on the outcome of this study there was no need to change the benefit-risk evaluation for Nobivac Myxo-RHD. The assessment was in favour of the product when used for the immunisation of pet rabbits including dwarf rabbits.

## *Environmental risk assessment*

The applicant has conducted an assessment of the potential risk to the environment from use of Nobivac Myxo-RHD Plus following the CVMP note for guidance EMEA/CVMP/074/95-Final.

The first phase of the assessment is provided, outlining that the potential exposure of the product to the environment and the level of risk associated with it is estimated as zero. Special attention was paid to the target animal species and in-contact target or non-target animals, the method of administration and the potential of excretion, and to which extent the vaccine harbours a related risk.

**Considerations for the environmental risk assessment:**

The likelihood that a hazard will occur is considered low as:

- Transmission to non-target species seems very unlikely as rabbits are the only host in Europe. As two main virulence factors (MGF and M11L genes) were disrupted from the myxoma genome, the applicant considers the constructs as non-pathogenic for the target species.

- No spread to non-target species like mice, chickens, and hares was demonstrated. RHDV2 may also infect some species of hares, but no reports of infection of European brown hares have arisen so far. A switch in host range is considered unlikely as the VP60 protein of RHDV2 does not become an integral part of the myxoma virus vector.
- Shedding and spread to unvaccinated rabbits was not detected for both vaccine strains. Field myxoma virus is disseminated via lymphocytes in the body of infected rabbits but vaccine strain 009 could not be detected in the blood of vaccinated animals.
- It has been demonstrated that strain 009 could be recovered from the eye of vaccinated rabbits when also infected with wild-type myxoma virus. However, strain 009 was shown unable to infect rabbits via the ocular or nasal route.
- Genetic stability was demonstrated for both vaccine strains by sequencing. However, parallel infection of vaccinated animals with field myxoma virus may result in a re-activation of the original myxoma strain used for the construct. Recombination of vaccine strain 009 with another live attenuated vaccine strain has been investigated and no adverse effects were observed. So far, no reports have been received that suggest a recombination of strain 009 and Shope fibroma virus.
- No risk to the environment is seen as the vaccine strains do not spread. Hence, the risk to the environment is negligible.
- Other effects like a change of target cells due to the VP60 insert from RHDV is not expected as in dissemination studies no vaccine virus could be isolated from liver cells representing the main target organ of RHDV.
- No toxic effects are expected from the ingredients of Nobivac Myxo-RHD Plus as no pharmacologically active components are included.
- The exceptional case that vaccine virus is transferred by biting insects from the injection site to another rabbit is highly unlikely. Hence, the chance that vaccine virus spreads through the rabbit population is negligible.

The environmental risk was properly assessed for vaccination with Nobivac Myxo-RHD Plus following the recommendations of guideline EMEA/CVMP/074/95.

Based on the data provided, the environmental risk assessment can stop at Phase I. Nobivac Myxo-RHD Plus is expected to pose a negligible risk to the environment when used as recommended.

## Environmental risk assessment for products containing or consisting of genetically modified organisms

Nobivac Myxo-RHD Plus falls within the scope of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms. Detailed information on the possible risks for humans and for the environment has been provided.

The final GMO has been shown to be phenotypically stable for 5 to 6 passages in rabbits. The genetic stability of strain 009 has been shown adequately. For strain MK1899 four point mutations were observed after five *in vivo* passages. Only two point mutations led to an amino acid exchange and one to a frame shift but these did not change the safety profile of the MK1899 strain. The VP60 of RHDV2 in strain MK1899 was inserted into the same site of the myxoma genome, using the identical flanking myxoma gene sequences and the same synthetic poxviral early/late promoter as for the insertion of VP60 of RHDV1 in strain 009. Information on indigenous vectors (biting insects) was provided. Therefore, the environmental risk assessment regarding GMOs is assessed to be effectively zero.

## *Overall conclusions on the safety documentation*

The safety of Nobivac Myxo-RHD Plus was investigated mostly in accordance with Commission Directive 2009/9/EC amending Directive 2001/82/EC, and the regulations for GMOs.

The application was accepted as MUMS because rabbits are a recognised minor species. As Nobivac Myxo-RHD Plus has a licensed precursor vaccine, i.e. Nobivac Myxo-RHD, which contains only one of the vaccine strains included in Nobivac Myxo-RHD Plus, several studies are included in this dossier that have already been provided for the dossier of this predecessor and were already assessed. This is acceptable according to guideline EMEA/CVMP/004/04 as it is considered that the insert of RHDV2 does not change the safety profile and characteristics of the new vaccine strain MK1899 compared to the already known strain 009 containing the same capsid structure protein of RHDV1.

The applicant performed two combined overdose and repeated dose studies to assess the safety of Nobivac Myxo-RHD Plus in commercial hybrid rabbits (New Zealand White) and pet rabbits (Dutch Belted). The vaccine did not cause clinical signs of myxomatosis or of RHD in any of the vaccinated rabbits, but local reactions at the injection sites were noted. The warnings included in the SPC are complete and adequate. No further macroscopic or microscopic lesions were observed attributable to the vaccine strains. Rectal temperatures were monitored and no or only a slight difference between vaccinated groups and controls was observed. In the SPC an adequate statement is included that temperature rises may occur after administration. A third combined study addressing overdose and repeated dose with Nobivac Myxo-RHD in dwarf rabbits is supplied. It was concluded that the product is safe in dwarf rabbits.

Reproductive safety was investigated in does vaccinated on the 7$^{th}$ day or the 21$^{st}$ day of pregnancy. The product was found to be safe when used in pregnant animals.

The product is not expected to adversely affect the immune response of the target animals or its progeny since the two main virulence factors, MGF and M11L, of myxoma virus have been deleted to insert RHDV1/2 protein VP60. Therefore, no suitable tests on the immunological functions were carried out.

The possible spread of the vaccine strains was evaluated during the combined overdose and repeated dose studies and no evidence of spread to in-contact control rabbits was observed. Additionally, based on existing evidence for vaccine strain 009, spread to non-target species is not expected. No dissemination studies were carried out with Nobivac Myxo-RHD Plus. Based on dissemination studies available for the vaccine strain 009, it is concluded that Nobivac Myxo-RHD Plus vaccine strains can be present in the skin at the injection site and disseminate to regional lymph nodes. Dissemination to other organs or shedding of the vaccine strains was not observed. However, dissemination of vaccine strain 009 to eyes was demonstrated when vaccinated dwarf rabbits were infected with wild-type myxoma virus at the time of or shortly after vaccination with Nobivac Myxo-RHD. Another study addressing not recommended routes of administration led to the conclusion that strain 009 is not able to infect rabbits via these routes.

Reversion to virulence was examined for each vaccine strain over 5 or 6 sequential rabbit passages; the results showed no evidence of an increase of virulence. Nevertheless, sequence analysis of the final passage showed 4 point mutations for strain MK1899, but these did not change the safety profile of the MK1899 strain.

The biological properties of the vaccine strains were described adequately and found to be acceptable. The recombination and the genomic re-assortment of the vaccine strains were also considered and it is concluded that the potential risk is low and acceptable. The simultaneous infection of a target cell with a wild-type myxoma virus and any of the vaccine strains may

theoretically result in the re-activation of the original myxoma virus used for the construction of the GMO. The same event may be possible with Shope fibroma virus. Both scenarios are considered as very unlikely.

The only risks concerning user safety are an accidental needle stick injury or a wound through damaged vaccine vials. Therefore, the potential health risk of the product to all users is considered low and acceptable when used in accordance with the SPC.

A withdrawal period of zero days is proposed for this vaccine as there are no hazards of possible residues.

No claims are made concerning use of Nobivac Myxo-RHD Plus with other vaccines and no specific studies have been provided.

No field trials are provided using Nobivac Myxo-RHD Plus, but four field studies (two in pregnant does and two with 5-week-old rabbits) and a post-authorisation safety field study performed with Nobivac Myxo-RHD are included in this dossier for support. These studies were intensively discussed during the marketing authorisation procedure for this product and several points in the SPC were adapted, which are also transferred to the SPC of Nobivac Myxo-RHD Plus. The results of the post-authorisation study confirmed the safety in pet rabbits.

In general, information concerning release of GMOs has been provided in appropriate studies. The vaccine virus strains have been shown to be phenotypically stable and in general genetically stable.

The vaccine is considered to be safe for the target species and non-target species, the user, the consumer and the environment.

# Part 4 – Efficacy

## *Introduction and general requirements*

Nobivac Myxo-RHD Plus is a live vaccine containing vectored myxoma viruses expressing capsid protein genes of classical and type 2 RHD viruses. It is the successor vaccine of Nobivac Myxo-RHD (centrally licensed in the EU in 2011) containing live myxoma-vectored RHD virus type 1 strain 009. Nobivac Myxo-RHD Plus was developed because shortly thereafter RHDV type 2 strains occurred in the field. Nobivac Myxo-RHD did not induce sufficient protection against these type 2 strains. Therefore, strain MK1899 was added to the composition of Nobivac Myxo-RHD. The minimum amount of the new strain MK1899 ($10^{3.0}$ FFU/dose) is the same as the licensed minimum amount of strain 009 in Nobivac Myxo-RHD.

As a consequence, rabbits are immunised against myxomatosis and rabbit haemorrhagic disease caused by classical RHD virus (RHDV1) and/or RHD type 2 virus (RHDV2). The main benefit of this approach is that the RHDV components can be grown *in vitro* instead of using live rabbits.

The vaccine is presented in freeze-dried form with accompanying solvent and is intended for rabbits from 5 weeks of age onwards (including pregnant does) to reduce mortality and clinical signs of myxomatosis and rabbit haemorrhagic disease (RHD) caused by RHDV1 and RHDV2. The minimum dose for each of the vaccine strains is $10^{3.0}$ FFU. The injection volume of one dose is 0.2 or 0.5 ml per rabbit, depending on the presentation. Onset of immunity is set at three weeks after the administration of a single subcutaneous dose. Yearly revaccination intervals are proposed.

Efficacy studies were carried out in young and adult animals, both under laboratory and field conditions. Unvaccinated animals served as controls. The influence of maternally derived antibodies

has been studied.

All trials have been performed as required by Directive 2001/82/EC as amended. The supporting field trials were conducted according to the principles of good clinical practice (GCP). Ph. Eur. monograph 1943 for live myxomatosis vaccines and relevant aspects of Ph. Eur. monograph 2325 for RHD inactivated vaccines were used as guidelines for efficacy, taking into consideration that the product falls within the scope of the MUMS guideline.

Since the use of laboratory animals should be avoided as much as possible and the minimum dose of vaccine virus strain 009 in Nobivac Myxo-RHD Plus is not lower than that of Nobivac Myxo-RHD (due to the addition of strain MK1899 the amount of myxoma vaccine virus has at least been doubled), the efficacy challenge studies against myxomatosis have not been repeated for Nobivac Myxo-RHD Plus. Consequently, the efficacy against myxomatosis is based on studies with Nobivac Myxo-RHD containing vaccine strain 009 only. In all studies performed with Nobivac Myxo-RHD Plus the antibody titres against myxoma virus were tested. Nearly all vaccinates developed high antibody titres against myxoma virus. The test method of the antibody testing was identical for all studies which were performed for the two products. For the duration of immunity studies a statistical comparison of the serological results obtained from the two vaccines was performed. No significant differences in the development and persistence of the antibody titres against myxoma virus could be detected.

Generally, this approach is considered acceptable, all the more as it is also supported by EU legislation (EU Directive 2010/63/EU on the protection of animals used for scientific purposes; EMA/CVMP/IWP/123243/2006-Rev.3 MUMS/limited market).

The efficacy testing against classical RHD, including onset and duration of immunity, was completely repeated for Nobivac Myxo-RHD Plus to rule out any interference from the type 2 RHDV capsid protein in the efficacy against classical capsid protein.

Field trials have not been performed with the product Nobivac Myxo-RHD Plus. According to EMA/CVMP/IWP/123243/2006-Rev.3 field trials are only required when adequate data from laboratory studies are not available. The applicant considers the laboratory data on efficacy (and safety) sufficient to conclude on the vaccine's efficacy in the target species, rabbits. Nevertheless, the applicant included the reports on the combined safety and efficacy field trials in the present documentation which were already submitted with the Nobivac Myxo-RHD documentation.

## *Challenge model:*

Four different challenge strains were used in the efficacy trials.

All were isolated from diseased rabbits or rabbits which had recently died on commercial farms in the UK, Spain or Italy where outbreaks occurred.

The isolated RHDV strains were passaged in young SPF rabbits. The challenge solution was obtained from the liver homogenate of these rabbits.

For each strain a study in rabbits was performed to determine the suitable challenge solution.

Detailed information on the challenge strains has been provided. The RHDV challenge was performed in the same way in every study. Each rabbit received challenge working solution, which was administered as follows: into each eye, into each nostril and orally.

For myxoma challenge studies the myxoma challenge material was administered by intradermal inoculation at 2 sites.

## *Efficacy parameters and tests:*

Requirements for demonstrating the efficacy of the vaccine as specified in the "Requirements for immunological veterinary medicinal products" (Title II, Part 7 of the Annex to Directive 2004/28/EC amending Directive 2001/82/EC), in Ph. Eur. monographs 5.2.7. "Evaluation of efficacy of veterinary vaccines", 0062 "Vaccines for veterinary use", 2325 "Rabbit haemorrhagic disease vaccine (inactivated)", and 1943 "Myxomatosis vaccine (live) for rabbits" were followed.

Challenge studies included vaccine groups and for every vaccine group a corresponding unvaccinated control group. After the challenge the rabbits were monitored for 14 days. Diseased animals, dead animals and rabbits euthanised for animal welfare reasons were documented. The results of the control and vaccine groups were evaluated according to the requirements of the specific Ph. Eur. monographs. The requirements of the RHD monograph were not met in every case because the RHD monograph cannot be regarded as completely applicable to RHDV2 virus.

In addition, blood sampling was performed on several occasions during the studies. The serum was tested for antibodies against myxoma virus (IFT) and RHDV1 and RHDV2 (haemagglutination inhibition [HI] test). The results of the vaccine group and control group were compared.

After RHDV challenge, liver samples were taken from dead and euthanised rabbits and the RHDV titre was tested (HA) in these samples. The results from the vaccination and control groups were compared.

The test parameters can be regarded as adequate. Validation studies for the methods used for antibody and antigen testing are not provided. For the kind of tests used, this is regarded as acceptable.

The same test methods, in particular the serological methods used for the Nobivac Myxo-RHD studies, were used for the evaluation of the Nobivac Myxo-RHD Plus studies.

## *Efficacy documentation*

To investigate the efficacy of Nobivac Myxo-RHD Plus 4 laboratory studies in young rabbits were conducted. A pilot vaccine batch containing the minimum antigen content per dose was tested. The onset of immunity of 3 weeks against RHDV1 and RHDV2 was tested in SPF New Zealand White (NZW) and SPF Dutch Belted rabbits (pet, but not dwarf breed). The duration of immunity of 12 months against RHDV1 and RHDV2 infections was investigated in NZW rabbits. The efficacy at the minimum vaccination age of 5 weeks was tested in the offspring of Nobivac Myxo-RHD Plus vaccinated mothers. Regarding myxoma virus, all rabbits in all 4 studies were tested for the development of antibodies only.

In addition, 6 laboratory efficacy studies and 4 field combined safety / efficacy studies were submitted. These studies were performed with Nobivac Myxo-RHD. In these studies, the onset of immunity of 3 weeks, the duration of immunity of 6 and 12 months was tested. Furthermore, a study to investigate the efficacy when vaccinating 5-week-old rabbits in the presence of traces of MDAs was conducted.

Two of the efficacy field studies were performed with pregnant does and 2 with the offspring of these does when vaccinated at the age of 5 weeks.

In the Nobivac Myxo-RHD studies challenges against myxoma virus and in some cases against RHDV1 (Ascot strain) were performed. In addition, antibodies against myxoma virus and RHDV1 were tested. In the laboratory trials, the rabbits were vaccinated with a vaccine dose containing the minimum antigen content.

Descriptions and/or SOPs regarding the methods used for the evaluation of serological and virus isolation results including their validity were provided.

## *Laboratory trials*

Overview of the submitted efficacy studies:

| *Study title* |
|---|
| Study to determine onset of immunity to RHDV of Nobivac Myxo-RHD Plus vaccine after subcutaneous administration in SPF rabbits |
| Study to determine onset of immunity to RHDV of Nobivac Myxo-RHD Plus vaccine after subcutaneous administration in pet rabbits |
| Study to determine efficacy of Nobivac Myxo-RHD Plus vaccine in MDA positive rabbits |
| Evaluation of the duration of immunity and booster response following vaccination with a new recombinant myxoma-RHDV vaccine |
| Evaluation of immunological responses to myxoma virus and RHDV in rabbits within twelve months after revaccination with a new recombinant MYXOMA-RHDV vaccine. |
| The efficacy of Myxo-RHD, in commercial rabbits, against myxoma virus challenge |
| Study to determine the efficacy of a myxoma/RHDV vaccine |
| Duration of immunity study (Myxomatosis/RHDV) |
| Six months duration of immunity study in commercial rabbits |
| Twelve months duration of immunity study in commercial rabbits |
| Study to determine the efficacy of Nobivac Myxo – RHD in MDA positive rabbits |
| A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in pregnant does (Milan farm) |
| A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in 5 weeks old rabbits (Milan farm) |
| A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in pregnant does (Valgrilla farm) |
| A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in 5 weeks old rabbits (Valgrilla farm) |

Explanation: The first 4 studies at the top of the table were performed with Nobivac Myxo-RHD Plus. The other studies were conducted with the precursor product Nobivac Myxo-RHD and were provided during the marketing authorisation procedure of this product

## *Dose determination*

The minimum efficacious vaccine dose has not been determined in separately conducted dose-finding studies for Nobivac Myxo-RHD Plus. The new vaccine strain MK 1899 was added to the composition of Nobivac Myxo-RHD. The minimum amount of $10^{3.0}$ FFU/dose is the same as the licensed minimum amount of the vaccine strain 009 in Nobivac Myxo-RHD. Due to the addition of strain MK 1899 the amount of myxoma vaccine virus has at least been doubled. As the minimum vaccine virus dose of strain 009 is not lower for Nobivac Myxo-RHD Plus than that for Nobivac Myxo-RHD, the dose determination is acceptable.

## *Onset of immunity*

### Myxoma virus

The studies demonstrating efficacy against myxomatosis by challenge were performed with Nobivac Myxo-RHD and have not been repeated for Nobivac Myxo-RHD Plus.

According to the applicant, the efficacy against myxomatosis will not be different compared to what has already been demonstrated for Nobivac Myxo-RHD. For animal welfare reasons it is not considered acceptable to perform additional myxomatosis challenge studies. EU Directive 2010/63/EU on the protection of animals used for scientific purposes is considered to be relevant; the MUMS status of the product is emphasised. Consequently, myxomatosis efficacy is based on challenge studies generated with the Nobivac Myxo-RHD vaccine containing strain 009 only.

However, in studies performed with Nobivac Myxo-RHD Plus antibody titres against the myxoma antigen are provided. Even though antibodies against myxoma virus do not correlate with protection, they are indicative of an immune response. Serological data and detailed method descriptions were submitted demonstrating comparability between the methods used and the results obtained with the two products.

For the duration of immunity studies, the antibody titres against myxoma virus from the studies performed with Nobivac Myxo-RHD and Nobivac Myxo-RHD Plus were statistically compared. No significant differences were detected between the studies performed with the two vaccines.

For demonstration of onset and duration of immunity the absence of efficacy challenge studies against myxomatosis for Nobivac Myxo-RHD Plus is normally not considered acceptable given the lack of correlation between antibody titres and protection. The applicant delivered a robust scientifically sound justification for not conducting new challenge studies with Nobivac Myxo-RHD Plus for demonstration of the vaccine's efficacy against myxoma virus infections. The presented challenge efficacy studies carried out with Nobivac Myxo-RHD (containing strain 009 at $10^3$ FFU/dose) are relevant and supportive of the efficacy against myxomatosis of Nobivac Myxo-RHD Plus at the proposed minimum virus content (strain 009 at $10^3$ FFU/dose plus strain MK1899 at $10^3$ FFU/dose). Any potential impact of the RHDV2 insert on the biological properties of the myxoma virus strain MK1899 in this context was discussed and can be neglected. In conclusion, there is no need for new challenge studies conducted with Nobivac Myxo-RHD Plus supporting the efficacy claim against myxomatosis.

For Nobivac Myxo-RHD two myxomatosis challenge studies are presented to demonstrate the onset of immunity of 3 weeks.

In the first study 4-5-week-old rabbits (New Zealand White) were divided into a vaccine group (11) and a control group (5). The vaccine group received one dose of the test vaccine with minimum antigen content. The controls remained untreated. Twenty-four days later, all were challenged with myxoma virus Precious strain by the intradermal route. A monitoring period of 25 days followed. All controls developed classical signs of severe myxomatosis and were euthanised on days 13 or 14 post challenge. All vaccinated animals survived but nearly every vaccinate received scoring points for clinical signs. This was mainly due to an increase in body temperature, but two rabbits also received higher scoring points due to skin swellings (not injection site). The statistical difference between the two groups was significant (P = 0.0022). The results are in line with a reduction claim against myxomatosis.

In the second study thirty-six New Zealand White female rabbits, 7-9 weeks of age, were divided into 4 groups (3 x 10 and 1 x 6 [seeders]). On day 0 of the study, all rabbits were bled.

Group 1 was administrated 1.0 ml of vaccine by the SC route of administration. The animals of Group 2 received an intradermal application of 0.1 ml of vaccine, the third group served as controls. On day 7, the 6 seeder rabbits were infected by the intradermal route with myxoma virus Precious strain. Nasal swab samples were collected from this group 4, 7, 9 and 11 days post infection. On day 9, a second bleeding of all animals was performed. Clinical monitoring of all groups started on day 11 (4 days after the infection of the seeders). On day 42, the study was terminated and the last blood samples were taken from the surviving animals.

All seeders and controls developed severe signs of myxomatosis. No vaccinated rabbit showed signs of myxomatosis over the whole study period.

From the two Nobivac Myxo-RHD Plus RHDV1 and RHDV2 challenge studies, antibody titres against myxoma virus are available (IFT method). The antibody titre development investigated by the same IFT method is comparable after vaccination with Nobivac Myxo-RHD and Nobivac Myxo-RHD Plus, in particular at the time of challenge. Even though antibodies against myxoma virus do not correlate with protection, they are indicative of an immune response.

All efficacy studies performed with Nobivac Myxo–RHD were already assessed during the previous licensing procedure. The minimum vaccination age, onset and duration of immunity including the outcome for the indication against myxomatosis are identical for Nobivac Myxo-RHD and Nobivac Myxo-RHD Plus.

As regards the Nobivac Myxo–RHD studies, no additional questions were asked and no different assessment was performed within the scope of the current procedure. It was only discussed and assessed whether such studies can be fully considered for the efficacy demonstration of Nobivac Myxo-RHD Plus.

## RHDV1 and RHDV2

Two RHDV challenge studies were performed with Nobivac Myxo–RHD PLUS. One study was performed in SPF New Zealand White rabbits and one in SPF Dutch Belted rabbits (pet but not dwarf breed).

In study**,** 60 female SPF New Zealand White rabbits at 5 weeks of age (except for the 15 rabbits of Groups 3 and 4, which were 9 weeks of age) were used. The rabbits were divided into 4 vaccination groups of 10 animals and 4 control groups of 5 animals. The rabbits of the vaccination groups received one dose (0.5 ml) SC in the scruff. The rabbits serving as controls were administered the same volume of Nobivac Solvent alone. Blood samples were taken several times including the vaccination and challenge day.

The following table gives an overview on the study design:

| Group | Quantity | Arrival age in weeks | Vaccination upon arrival at study day | Inoculum | Study day of challenge | Challenge strain |
|---|---|---|---|---|---|---|
| 1 | 10 | 5 | 0 | Myxo-RHD PLUS | 49 | RHDV 1 Ascot |
| 2 | 5 | 5 | 0 | Solvent | 49 | RHDV 1 Ascot |
| 3 | 10 | 9 | 28 | Myxo-RHD PLUS | 49 | RHDV 1 Ascot |
| 4 | 5 | 9 | 28 | Solvent | 49 | RHDV 1 Ascot |
| 5 | 10 | 5 | 28 | Myxo-RHD PLUS | 49 | RHDV2 Spain-Aixala |
| 6 | 5 | 5 | 28 | Solvent | 49 | RHDV2 Spain-Aixala |
| 7 | 10 | 5 | 28 | Myxo-RHD PLUS | 49 | RHDV2 Italy2 |
| 8 | 5 | 5 | 28 | Solvent | 49 | RHDV2 Italy2 |

Animals from group 1 and 2 were vaccinated at the age of 5 weeks. Animals from group 3 and 4 were vaccinated at the age of 9 weeks.

Challenge against RHDV1 was performed with challenge strain RHDV1 Ascot in all groups on day 49 of the trial when the animals were 12 weeks of age. This is acceptable because it is known that young rabbits are not or not fully susceptible to RHDV1 infections. Therefore, a challenge at a younger age is not advisable. To perform the RHDV1 challenge not before the age of 12 weeks, even if it does not fit the minimum vaccination age and the onset of immunity, has been accepted in former RHDV1 licensing procedures including Nobivac Myxo-RHD. This study design was justified as follows: Nine-week-old animals were included because rabbits are not yet sensitive to classical RHDV1 at 8 weeks of age (age of challenge for a 3 weeks onset of immunity when vaccinated at 5 weeks of age). Nine-week-old animals were challenged with classical RHDV1 strain Ascot at three weeks after vaccination and 5-week old animals at seven weeks after vaccination. This approach is supported and is regarded as valid to demonstrate a 3 week onset of immunity for the RHDV1 virus.

Animals from group 5 to 8 were vaccinated at the age of 5 weeks.
On day 49 of the study, at the age of 8 weeks, animals were challenged with RHDV2 strain Spain Aixala (Group 5+6) or RHDV2 strain Italy 2 (Group 7+8) respectively.

The phenomenon of natural protection of young rabbits does not occur for RHDV2 strains. Therefore, the rabbits were challenged 3 weeks after vaccination at the age of 5 weeks.

All rabbits of each control group died or were euthanised for animal welfare reasons within 3 days after the challenge.

All rabbits except for 3 from the vaccination groups survived the challenge without showing any signs of disease. These three rabbits did not develop any antibodies. As also some rabbits of the duration of immunity study did not respond serologically after the first vaccination and the revaccination one year later, it is more likely that several rabbits used in the studies were non-responders.

Two of these animals interpreted as non-responders belong to the RHDV2 Spain Aixala vaccination group. Therefore, the protection result of this group is 80 % and a reduction in mortality was demonstrated for RHDV 2.

In addition, it is remarkable that the animals were protected in the challenge despite the very low antibody titres induced against RHDV1 and RHDV2 in all vaccinates in the studies performed with Nobivac Myxo-RHD Plus.

All rabbits from the 3 vaccination groups survived the challenge. All rabbits belonging to the control groups died or were euthanised for animal welfare reasons within 3 days after the challenge. All vaccinated rabbits showed vaccine-induced antibodies on a low level.

With these studies the applicant confirmed onset of immunity of 3 weeks against RHDV1 and RHDV2.

## *Duration of immunity*

### RHDV1 and RHDV2

One study, conducted with Nobivac Myxo-RHD Plus, is presented to demonstrate the duration of immunity of 12 months and a sufficient booster effect after a revaccination at 12 months after the primary vaccination. In this study challenges were performed against RHDV1 and RHDV2.

Two submitted studies (see below) were conducted with Nobivac Myxo-RHD, one for the demonstration of immunity of 6 months and one for 12-month duration, together with a sufficient booster effect for the revaccination. In both studies challenges with myxoma virus were performed.

The first study was performed with Nobivac Myxo–RHD PLUS under blinded conditions. Female NZW rabbits of 5 weeks of age were used. The rabbits were allocated into 4 vaccination groups of 12 rabbits each and 4 control groups of 7 rabbits each (the relevant Ph. Eur. monograph requests 10 vaccinates and 5 controls). The vaccination groups were vaccinated SC into the scruff with one dose containing $10^{2.4}$ FFU/dose Myxo-RHDV1 and $10^{2.5}$ FFU/dose Myxo-RHDV2. This is less than the minimum virus content of $10^{3.0}$ FFU for both vaccine strains per dose. Before the vaccination, the vaccine was erroneously diluted to the lower virus content.

Blood samples were taken before vaccination and on days 21, 42, 90, 140, 196, 252, 308 and 368 (prior to challenge) of the study. Blood samples were tested for antibody titres against RHDV1, RHDV2 (HI method) and myxoma virus (IFT method).

After 12 months, 3 vaccine groups and the corresponding control groups were challenged either with RHDV1 Ascot strain (T2 and T5), RHDV2 Italy2 strain (T1 and T4), or with RHDV2 Spain Aixala strain (T3 and T6). The last vaccine group (T7) received a booster vaccination containing the minimum antigen content and had blood sampled together with the corresponding control group (T8) again 3 weeks later.

Four vaccinated animals did not develop antibodies against the vaccine at all. Prior to challenge these rabbits were transferred to the booster group.

The rabbits from the control groups, except one animal, did not develop vaccine-specific antibodies over the whole year. The vaccinates developed antibodies against myxoma virus, RHDV1 and RHDV2. The antibody titres increased for up to 6 weeks and nearly stayed on this level with only a little decrease until challenge. The titres were markedly higher compared to the onset of immunity studies. No difference in the detection method is reported. One rabbit belonging to group T1 (vaccinates) was tested positive as late as on day 90 as well as 3 rabbits from group T3 (vaccinates), which were tested positive as late as on day 42. All showed positive titres afterwards until challenge.

All vaccinated rabbits that were challenged with RHDV1 survived challenge, while for each of the RHDV2 challenges 1 vaccinated rabbit did not survive challenge. For RHDV1 only 3 out of the 7 controls did not survive challenge. This may be caused by the challenge model, which was developed in young rabbits and not in animals of over one year of age. These animals may have more age resistance to RHDV and as a result some of the controls survived challenge. All control animals died after challenge with strain Italy2 while 5 out of 7 controls died after challenge with strain Spain-Aixala. Strain Spain-Aixala is an early RHDV2 isolate which is not as virulent as the more recently isolated RHDV2 strains like strain Italy2.

Between days 5 and 11, two additional rabbits from each of the groups T1, T3 and T6 died or were euthanised. These rabbits showed diarrhoea, lethargy and dysbiosis in the post mortem examination; in addition, only very low RHDV titres could be detected in the post mortem liver samples. High RHDV titres in these samples would indicate a severe RHDV infection. The applicant assessed these signs as not challenge-related and excluded these animals from the evaluation.

The statistical evaluation of the mortality and clinical signs after challenge was performed and for the RHDV1 strain no significant difference between vaccination group and control group could be shown.

The serological results detected 3 weeks after the revaccination (booster) reached the titre detected after the primary vaccination against myxoma virus. The applicant indicated that the booster group and the corresponding control were transferred into a follow-up study, which is summarised below. As already mentioned above, some rabbits, which were seronegative after the first vaccination and transferred into the booster group, and two of these rabbits remained seronegative again after the

revaccination. As all SPF New Zealand White rabbits were ordered from the same provider, it is likely that the seronegative rabbits from the vaccination groups of the onset of immunity studies were non-responders as well. Serological results of animals in the follow-up study showed titres against all three components, which remained stable for one year after revaccination.

The results of the duration of immunity challenge study allow a reduction claim for the RHDV 1 and 2 vaccine component.

The follow-up study included an additional RHDV1 challenge, which sufficiently demonstrates duration of immunity of one year after booster vaccination against RHDV1. Briefly, 23 rabbits at 14 months of age were included in the study. Sixteen (16) animals were already vaccinated in the previous study (described above) at 5 weeks of age (primary vaccination) and re-vaccinated 12 months later (group T1 and T2). Seven control rabbits from the previous study which had only received solvent were also used as controls in this study (group T3). The 16 vaccinated rabbits were divided into two groups based on their antibody responses to RHDV and myxoma virus. Eleven animals which developed a "normal" range of antibodies against RHDV1, RHDV2 and myxoma virus after primary vaccination were allocated to group T1. 5 rabbits that had no antibodies to RHDV1, RHDV2 and myxoma after primary vaccination were allocated to group T2. One rabbit of group T1 and one rabbit of the control group (T3) had to be euthanised for animal welfare reasons after the start of the study. The reasons for euthanasia were unrelated to vaccination (leg injuries). 12 months after revaccination (rabbits were 2 years of age) two animals of group T1, four animals of group T2 and six animals of the control group were challenged with RHDV1 strain Ascot (classical strain). That means mainly the poor or non-responders to the vaccination(s) (group T2) have been used for the RHDV 1 challenge. In vaccinated rabbits clinical signs were only observed in 2 out of 6 animals of which one animal belonged to the non-responder group (T2). The animal belonging to group T1 recovered the day after whereas the rabbit belonging to group T2 was euthanised for animal welfare reasons on day 3 after challenge. In contrast to the RHDV1 challenge performed in the previous DOI study, all control rabbits died or were euthanised for animal welfare reasons within 5 days after the RHDV1 challenge. Furthermore, a significant difference in mortality rates was observed between vaccinated and control rabbits.

The following table summarizes mortality after RHDV-1 Ascot challenge:

| **Challenge virus** | **Inoculum** | **Group (no. rabbits)** | **Survival (%)** | **Death (%)** | **P values vaccinates vs. controls*** |
|---|---|---|---|---|---|
| RHDV1 Ascot | Myxo-RHD Plus | T1 + T2 (6) | 83 | 17 | 0.0152* |
| | Nobivac Solvent | T3 (6) | 0 | 100 | |

*Statistical difference in mortality after RHDV1 challenge between vaccinated and control rabbits (Fisher's Exact test)

The surviving rabbits of the vaccination groups were euthanised on day 6 after the challenge. Post mortem liver and spleen samples were taken and tested for RHDV titre in the organs. Elevated titres indicate that the animal suffered or died due to RHDV infection or may develop signs of illness shortly. Only in the liver of 1 surviving vaccinated rabbit a marked RHDV titre was detected after euthanasia. This rabbit might have developed clinical signs of RHDV1 infection after day 6 post challenge when the surviving rabbits were euthanised because the observation period was limited to

5 days post challenge. But even if this rabbit were calculated as second case of death from the vaccination group, this would not change the final conclusion of the challenge results.

Therefore the applicant's conclusion that duration of immunity of one year for RHDV1 after re-vaccination has been shown to be acceptable.

In summary, the results of this study support a duration of immunity against RHDV1 according to the indication of one year after a second vaccination and stable antibody titres for one year against all three components after revaccination.

**Myxoma virus**

As mentioned above, the applicant submitted two studies that were already part of the Nobivac Myxo-RHD dossier.

**DOI 6 months**

The study was performed to confirm the duration of immunity of 25 weeks against myxomatosis in commercial rabbits. Fifteen rabbits were vaccinated with the minimum dose of Nobivac Myxo-RHD. Seven rabbits were used as unvaccinated controls. The myxomatosis challenge nearly 6 months later was performed by placing myxoma virus-infected shedder rabbits into the groups. Eighty-six percent of the rabbits from the vaccination group were fully protected. Serology for RHDV and myxoma virus was performed.

**DOI 12 months**

Twenty rabbits were vaccinated with the minimum dose of Nobivac Myxo-RHD. Ten other rabbits remained as unvaccinated controls. Twelve months after vaccination, 10 vaccinated and 5 control rabbits were challenged against myxomatosis. All controls developed myxomatosis; some vaccinates showed minor clinical signs of myxomatosis.

Thirteen months after vaccination, the remaining 10 vaccinates and 5 controls were challenged with RHDV1 Ascot strain. No vaccinate showed clinical signs of disease while all controls died within 60 hours after the RHD challenge.

From these studies it was concluded that the DOI of 12 months according to the indication had been demonstrated for Nobivac Myxo-RHD.

## *Maternally derived antibodies (MDA)*

Two studies have been submitted; one was performed with Nobivac Myxo-RHD Plus and the second study with Nobivac Myxo-RHD. The second study was also part of the Nobivac Myxo-RHD dossier.

In the first study offspring of mothers vaccinated with Nobivac Myxo-RHD Plus and offspring of unvaccinated does were used. The mothers had been used for another safety study. Vaccination was performed one week after mating.

Twelve rabbits from vaccinated mothers were divided into groups 2 and 3, and 6 rabbits from unvaccinated mothers were assigned to group 1.

The rabbits were bled at the age of 4 weeks (pre-screening of the whole litter for the selection of the rabbits showing the highest RHDV antibody titres), 5 weeks and 8 weeks, respectively.

Groups 1 and 2 received a vaccination with a minimum dose of Nobivac Myxo-RHD Plus at the age of 5 weeks.

All rabbits were tested negative on Day 0 (5 weeks of age); residual MDAs against RHDV and myxoma virus could not be detected.

| | | | HI titre to RHDV1 $log_2$ | HI titre to RHDV2 $log_2$ | IFN titre to myxoma virus $log_2$ |
|---|---|---|---|---|---|
| Group | MDA status | inoculum | Day 21 | Day 21 | Day 21 |
| 1 | MDA- | vaccine | 3.7 | 3.8 | 12.3 |
| 2 | MDA+ | vaccine | 2.3 | 2.7 | 11.8 |
| 3 | MDA+ | solvent | negative | negative | negative |

The serological RHDV1 and RHDV2 results obtained in the MDA+ vaccination group are low and it is not clear if the rabbits would be sufficiently protected against RHDV1 or RHDV2 for 12 months. Challenges were not performed in this study.

The mothers of the MDA-positive offspring were vaccinated one week after mating. The onset of immunity for the vaccine is three weeks. That was 2 days before parturition. Due to the rabbits' placenta type, a part of the maternally derived antibodies (in this case IgG) are already transmitted diaplacentally during pregnancy. It could be clarified that pregnant does are able to provide MDAs via placenta or via colostrum comparable to does which are vaccinated already a few weeks before mating. Therefore, the animals used can be assessed as suitable for this study. The outcome of the serological testing 3 weeks after vaccination revealed differences in the mean group titres between the offspring of the vaccinated does and the unvaccinated does. The antibody titres in the offspring-group of vaccinated does were on a very low level. In this case, there are doubts if a single vaccination at this age would provide 12-months duration of immunity.

Since it could not be concluded that there is no impact on the efficacy of Nobivac Myxo-RHD Plus when administered to animals with maternally derived antibodies at the minimum age recommended for vaccination. Therefore the following warning was included in the SPC section 4.4:

"High levels of maternally derived antibodies against myxoma virus and/or RHD virus can potentially reduce the efficacy of the product. To ensure the full duration of immunity, vaccination from 7 weeks of age is advised in this case." This warning can be regarded as adequate.

In the second study, the offspring from the tenfold overdose safety study in pregnant does was used (Nobivac Myxo-RHD study). Groups of rabbits were vaccinated with one minimum dose of Nobivac Myxo-RHD at the age of 4, 6 and 8 weeks. Six-week-old animals from the same litters served as unvaccinated controls. Blood samples for antibody detection were collected. Three weeks after vaccination a challenge against myxomatosis was performed (myxoma virus strain Precious). Rabbits vaccinated at the age of 6 and 8 weeks were protected. Some of the rabbits from the 4 week vaccination group developed signs of myxomatosis after the challenge.

Although an RHDV challenge was not carried out, a serological analysis showed that those rabbits which responded to myxoma virus after vaccination also responded with an anti-RHDV1 response.

This study demonstrates that the offspring of vaccinated mothers can be vaccinated successfully under field conditions with Nobivac Myxo-RHD at the age of 6 weeks and older. This study can be regarded as informative only for this application, because the requested minimum vaccination age for Nobivac Myxo-RHD Plus is 5 weeks of age.

## *Interactions*

No studies investigating the efficacy of Nobivac Myxo-RHD Plus when administered together with other veterinary medicinal products at the same time have been submitted.

Therefore, the wording of SPC section 4.8 is regarded as adequate.

## *Field trials*

Field trials have not been performed with the product Nobivac Myxo-RHD Plus.

Instead, field studies which were performed with Nobivac Myxo-RHD were re-submitted. For MUMS products, conduct of field studies is not obligatory.

In total, three combined safety and efficacy field trials have been carried out with Nobivac Myxo-RHD at two different locations using 420 animals. For Nobivac Myxo-RHD a fourth study was included, but in this study a placebo group, which also acted as control group for challenge, was erroneously vaccinated. As a result, the myxomatosis challenge part of this study can be considered as invalid and, therefore, this study is not included in this part of the dossier but in Part 3.

A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in pregnant does:

This study was intended to be performed in non-vaccinated animals to investigate the safety and efficacy of Nobivac Myxo-RHD in myxoma virus antibody-negative pregnant does. When serology data were available, it became obvious that the animals had been vaccinated against myxomatosis before. The study was not stopped but continued to gain information on the use of Nobivac Myxo-RHD in rabbits which were vaccinated some time before with a commercial vaccine against myxomatosis.

Fifteen does were vaccinated (titre $10^{4.5}$ pfu/ml) on day 13 and another 15 does were vaccinated on day 19 of pregnancy with one dose of Nobivac Myxo-RHD. A further 15 does serving as control group were treated with the stabiliser (virus-free) of Nobivac Myxo-RHD on day 13 or day 19 of pregnancy. Antibody titres against myxoma virus and RHDV1 were determined in pre- and post-vaccination serum samples of the does and in their offspring (MDAs) at eight days and five weeks of age. The does were assessed for local (daily from 1 day before administration until 14 days after administration) and systemic (daily clinical observations) reactions. Kindling results and mortality of the progeny were recorded until weaning to assess any effects of vaccination on reproductive performance.

The results showed that the SC administration of Nobivac Myxo-RHD to pregnant does was safe. Vaccination of rabbits which have been exposed to myxoma virus before vaccination with Nobivac Myxo-RHD may not elicit an antibody response against RHDV1 as a result of immunity against the vector.

A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in 5-week-old rabbits:

To assess the efficacy of Nobivac Myxo-RHD, NZW rabbits of 5 weeks of age were injected SC with Nobivac Myxo-RHD or a placebo in a volume of 0.2 ml. Antibody titres against RHDV1 and myxoma virus were determined at 5, 8 and 12 weeks of age. Five weeks after the vaccination the rabbits were challenged with myxoma virus. Twelve weeks after the vaccination a RHDV1 challenge was performed at the challenge facility. Unvaccinated SPF rabbits of the same breed served as additional controls.

Challenge with myxoma virus:

All SPF rabbits died or were euthanised with signs of myxomatosis. The same was recorded for 11/12 from the "farm" control group. The surviving animal from this group showed signs of myxomatosis as well. 3/12 of the vaccination group died with signs of myxomatosis (25%), nearly all other rabbits of the vaccination group developed mild signs of myxomatosis.

Challenge with 2 different RHDV1 virus strains:

100% mortality occurred in the SPF control and in the "farm" control groups. 1/12 (8%) vaccinated animal died after infection with the BS89 strain and 3/12 (25%) vaccinated animals died after challenge. Only in one of these animals RHDV could not be detected in post mortem liver or spleen tissue samples. The protection rates in the vaccination groups are significantly higher compared to the controls.

A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in pregnant does:

The objective of the study was to investigate the safety and efficacy of Nobivac Myxo-RHD in pregnant does. Forty adult (parity >1) does, which had been vaccinated in the past against myxomatosis and RHD using commercially available vaccines, and 20 unvaccinated young does (parity =0) were selected for the study. Fifteen of these animals were SC vaccinated with a single 1 ml dose of the reconstituted vaccine, titre $10^{4.5}$ pfu/ml, on day 14 and 15 animals on day 19 of pregnancy, while the remaining 30 animals were injected with 1 ml of a placebo.

Serology (myxoma virus and RHDV1 antibodies by IFT and HI, respectively) was performed pre- and post-vaccination. The progeny was examined in view of maternally derived antibodies against myxoma virus and RHDV1 at 9 days and 5 weeks of age. Serology was also used to monitor any spread of the vaccine virus from vaccinated to unvaccinated rabbits.

A serological response against both myxoma virus and RHDV1 was obtained in the vaccinated animals, irrespective whether the does had been vaccinated or not vaccinated before the start of the study. However, the antibody titres against myxoma virus were not significantly increased in the adult does. This was probably due to the fact that these animals had been vaccinated against myxomatosis in the past.

MDAs against both myxoma virus and RHDV1 were measured in the progeny at 9 days of age. These MDAs declined to non-detectable or low levels by 5 weeks.

A field trial in Italy to investigate the safety and efficacy of Nobivac Myxo-RHD in 5-week-old rabbits:

This trial was carried out to confirm the safety and efficacy of Nobivac Myxo-RHD under field conditions. As the time and level of field infections are not predictable, some study animals were challenged with myxoma virus and some with RHDV1 under laboratory conditions.

NZW rabbits of 5 weeks of age were injected with the vaccine in a volume of 1 ml or 0.2 ml or with a placebo in a volume of 1 ml. As additional controls, SPF rabbits of the same breed were used. Due to an error the placebo group was also vaccinated and is indicated as "placebo/myxo" group. Antibody titres against RHDV1 and myxoma virus were determined at 5, 8 and 12 weeks of age. Serology was also used to monitor potential spreading capacity of the vaccine from vaccinated to unvaccinated rabbits. Five weeks after the vaccination the rabbits were challenged with virulent myxoma virus. Twelve weeks after the vaccination a RHDV1 challenge was performed at the challenge facility. Unvaccinated SPF rabbits served as controls.

The vaccinated rabbits developed antibody titres against both myxoma virus and RHDV1. The serological results in unvaccinated rabbits showed that Nobivac Myxo-RHD did not spread and that field infections during the study period were absent.

During the challenge experiments all SPF controls died (0% survival). The vaccinated rabbits, however, were well protected and had a survival rate of 74% (myxomatosis) and 88% (RHD), respectively.

As the mortality rate of the vaccinated rabbits was significantly lower compared to the unvaccinated SPF controls, the results of this study were in line with the claim in the Nobivac Myxo-RHD SPC as required: "…..to reduce mortality and clinical signs of myxomatosis and to prevent mortality due to RHD caused by classical RHD virus strains".

## *Overall conclusion on efficacy*

To demonstrate the efficacy according to the indication in combination with the minimum vaccination age the applicant has presented 4 laboratory studies performed with Nobivac Myxo-RHD Plus. In addition, all efficacy studies performed with the already licensed vaccine Nobivac Myxo-RHD are part of this dossier again. This vaccine is the corresponding product containing the live myxoma vectored RHDV1 strain only.

The focus of the Nobivac Myxo-RHD Plus studies was to demonstrate the onset of immunity of 3 weeks for meat or pet breed rabbits, the efficacy in the offspring of vaccinated does at 5 weeks of age, and the duration of immunity of 12 months with a sufficient booster effect for the vaccination given 1 year later. In order to demonstrate the efficacy, challenges were performed against one RHDV1 and two RHDV2 strains. In addition, antibody titres were monitored. No myxoma virus challenge studies were performed with Nobivac Myxo-RHD Plus. From the antibody titres alone it is normally not possible to deduce a reliable protection. However, challenge studies against myxomatosis were performed with Nobivac Myxo-RHD with satisfactory results. This is acceptable because one dose of Nobivac Myxo-RHD Plus contains a complete dose of Nobivac Myxo-RHD together with the same amount of virus of the myxoma vectored RHDV2 vaccine strain. Serological data and detailed method descriptions were made available. There were no changes in the test methods used in the Nobivac Myxo-RHD Plus studies compared to the earlier performed Nobivac Myxo-RHD studies. This underlines the comparability between the results obtained with the two products as regards myxomatosis and RHDV1. A statistical analysis of the serological data was provided demonstrating satisfactorily that no negative effect on the antibody responses against myxoma virus occurs by the addition of the myxoma vectored RHDV2 vaccine strain.

The applicant submitted a scientifically sound justification that the presented challenge efficacy studies carried out with Nobivac Myxo-RHD are relevant and also fully supportive of the efficacy against myxomatosis for the Nobivac Myxo-RHD Plus vaccine.

Regarding the studies performed with Nobivac Myxo-RHD Plus all major questions and other concerns could be solved, including the sufficient demonstration of the duration of immunity for 12 months of the RHDV1 vaccine component after re-vaccination. The onset of immunity for the RHDV1 vaccine component was satisfactorily clarified by the applicant.

The approved indication as well as the onset and duration of immunity for the myxomatosis and RHDV2 vaccine components can be regarded as sufficiently supported by the studies.

From the data provided it can be concluded that the vaccine is able to induce sufficient protection against all three components according to the indication over a period of one year when re-vaccinated one year after the primary vaccination.

There were doubts whether there is still a negative impact of the vaccination for the offspring of vaccinated does at the minimum vaccination age of 5 weeks. Therefore the applicant accepted a warning in SPC section 4.4 that high levels of maternally derived antibodies can reduce the efficacy of the vaccine and in such cases the vaccination should be performed at 7 weeks of age:

"For active immunisation of rabbits from 5 weeks of age onwards to reduce mortality and clinical signs of myxomatosis and rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV1) and RHD type 2 virus (RHDV2)."

# Part 5 – Benefit-risk assessment

## *Introduction*

Nobivac Myxo-RHD Plus is a live attenuated, lyophilised vaccine for active immunisation of rabbits from five weeks of age by one subcutaneous injection to reduce mortality and clinical signs of myxomatosis and to prevent rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV1) and RHD type 2 virus (RHDV2).

The active substances are two myxoma-vectored RHD virus strains where the virulence factors MGF (myxoma growth factor) and the M11L (required for myxoma replication in lymphocytes) genes have been deleted and instead a capsid protein gene from RHDV1 or RHDV2 has been inserted. Strain 009 contains the VP60 protein of RHDV1 and vaccine virus strain MK1899 the VP60 protein of RHDV2.

No adjuvant or preservatives are included in this product. The solvent Nobivac Solvent used for Nobivac Myxo-RHD Plus is already used for the applicant's currently authorised live rabbit vaccine Nobivac Myxo-RHD.

The dossier was submitted in line with requirements of Article 12(3) of Directive 2001/82/EC. The product has been classified as MUMS/limited market and therefore reduced data requirements apply that have been considered in the assessment.

The evaluation follows Guideline EMEA/CVMP/248499/2007 (Recommendation on the evaluation of the benefit-risk balance of veterinary medicinal products).

## *Benefit assessment*

## Direct therapeutic benefit

Myxomatosis is a lethal, generalised viral disease of the European rabbit (*Oryctolagus cuniculus*) caused by myxoma virus, a member of the Poxviridae family. Rabbit haemorrhagic disease (RHD) is a highly contagious and acute fatal hepatitis of the European rabbit, caused by a calicivirus (genus Lagovirus). Since 2010, in addition to the classical variant RHDV1, a further variant of RHDV was identified, called RHDV2.

Controlled clinical trials demonstrated that the product is efficacious. The following SPC claims are approved:

For active immunisation of rabbits from 5 weeks of age onwards to reduce mortality and clinical signs of myxomatosis and rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV1) and RHD type 2 virus (RHDV2).

Onset of immunity: 3 weeks.

Duration of immunity: 1 year.

## Additional benefits

- The vaccine strains can be grown in vitro using a continuous cell line of rabbit kidney cells. Therefore, it is not necessary to use the target species for the production of the vaccine antigen RHDV components.
- No adjuvant or preservatives are added to Nobivac Myxo-RHD Plus.
- An easier vaccination schedule for industrial rabbit farms is possible.
- Safe for use in rabbits from 5 weeks of age.
- Safe for use in pregnant does.
- No excretion of the vaccine strain and no spread to unvaccinated rabbits or non-target species were detected.

## Risk assessment

Quality:

The starting materials, production process and final product are considered to be of adequate quality.

Safety:

The main potential risks are identified as follows:

For the target species and non-target species:

Subcutaneous injection of Nobivac Myxo-RHD Plus induces small, soft, painless swellings at the injection site which completely disappear within 3 weeks. In pet rabbits, local reactions at the injection site such as necrosis, scabs, crusts or hair loss may occur in very rare cases.

Serious hypersensitivity reactions, which may be fatal, may occur after vaccination in very rare cases.

The appearance of mild clinical signs of myxomatosis may occur within 3 weeks of vaccination in very rare cases. Recent or latent infection with field myxoma virus seems to play a role in this to a certain extent.

Studies have been carried out in pet rabbits including dwarf rabbits indicating that the vaccine is safe in these breeds of rabbits.

No vaccine-related negative effects were noted in pregnant does vaccinated on day 7 and on day 21 of pregnancy.

For the user:

There are no user and consumer safety issues.

For the environment:

As regards the assessment, in accordance with Directive 2001/18/EC, all points to be considered for a live recombinant vector vaccine have been addressed. In general it is considered that the GMO poses no enhanced risk for target and non-target animals, environment and humans since the vaccine virus is only found at the injection sites and in special cases in the eyes of vaccinated

rabbits but does not spread to unvaccinated rabbits.

## *Risk management or mitigation measures*

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user, environment and consumer and to provide advice on how to prevent or reduce these risks.

## *Evaluation of the benefit-risk balance*

Nobivac Myxo-RHD Plus has been shown to actively immunise rabbits from 5 weeks of age onwards to reduce mortality and clinical signs of myxomatosis and rabbit haemorrhagic disease (RHD) caused by classical RHD virus (RHDV1) and RHD type 2 virus (RHDV2). The onset of immunity is set at 3 weeks, the duration at 1 year.

The formulation and manufacture of the product are well described and specifications set will ensure that a product of consistent quality will be produced.

It is well tolerated by the target animals and presents a negligible risk for users and the environment and appropriate warnings have been included in the SPC.

## *Conclusion*

Based on the CVMP review of the data on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for Nobivac Myxo-RHD Plus is approvable. The CVMP considers that the benefit-risk balance of the application is positive and, therefore, recommends the granting of the marketing authorisation of the above mentioned veterinary medicinal product.