22 October 2020
EMA/565735/2020
Veterinary Medicines Division

## Committee for Medicinal Products for Veterinary Use

# CVMP assessment report for Nobivac DP Plus (EMEA/V/C/005251/0000)

Vaccine common name: Canine distemper vaccine (live, attenuated) and canine parvovirus 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 31 October 2019 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Nobivac DP Plus, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

On 7 October 2020, the CVMP adopted an opinion and CVMP assessment report.

The eligibility to the centralised procedure was agreed upon by the CVMP on 6 December 2018 as Nobivac DP Plus has been developed by recombinant DNA technology.

The applicant applied for the following indication which is considered appropriate by CVMP: For the active immunisation of puppies from 4 weeks of age onwards to prevent clinical signs and mortality of canine distemper virus (CDV) infection and canine parvovirus (CPV) infection and to prevent viral excretion following canine distemper virus infection and following canine parvovirus infection.

Onset of immunity: for canine distemper virus: 7 days;
for canine parvovirus: 3 days.

Duration of immunity: 8 weeks.

The active substances of Nobivac DP Plus are canine distemper virus (live, attenuated) and canine parvovirus (live recombinant), to provide early protection against CDV and CPV and the immunity against CPV is achieved in animals of 4 weeks of age with levels of maternal antibodies in the highest range present at this age. The target species are dogs (puppies). The product is intended for administration by the subcutaneous route.

Furthermore, the CVMP considers that the live recombinant canine parvovirus strain 630a is a new active substance, as claimed by Intervet International B.V.

Nobivac DP Plus, lyophilisate and solvent for suspension for injection contains $10^{5.1}$ – $10^{6.5}$ $TCID_{50}$/dose canine distemper virus strain Onderstepoort and $10^{5.1}$ – $10^{6.7}$ $TCID_{50}$/dose canine parvovirus strain 630a (live recombinant) and is presented in packs containing:

- 5 x 1 dose vial of vaccine and 5 vials containing 1 ml of solvent
- 25 x 1 dose vial of vaccine and 25 vials containing 1 ml of solvent

The rapporteur appointed is Esther Werner and the co-rapporteur is Frida Hasslung Wikström.

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

## *Scientific advice*

The applicant requested scientific advice from the CVMP on 14 October 2016 and it was provided on 16 February 2017 (EMA/CVMP/SAWP/724500/2016). The scientific advice pertained to the quality and the safety part of the dossier.

The questions discussed in the advice concerned a new reversion to virulence study for the CDV strain, whether the plasmid preparation would be considered as a chemical starting material, and the extraneous agents and mycoplasma testing of the plasmid preparation.

It was concluded that a new study on reversion to virulence is not necessary. However, a respective justification and risk assessment was requested. This is provided in Part 3 of the dossier.
Testing of the plasmid preparation for extraneous agents and mycoplasma was also considered dispensable provided a satisfactory risk assessment is available. The document is provided in Part 2.

The company's answers on the questions regarding the plasmid as a starting material were satisfactory.

### *MUMS/limited market status*

Not applicable.

# Part 1 - Administrative particulars

### *Detailed description of the pharmacovigilance system*

A detailed description of the pharmacovigilance system (version 3.0, dated 1st July 2018) which fulfils the requirements of Directive 2001/82/EC was provided. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

### *Manufacturing authorisations and inspection status*

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

Production of the active substances is performed by Intervet International B.V. in Boxmeer, Netherlands.

Manufacture of finished product and primary packaging (filling and freeze-drying) was initially planned to be performed at a CMO. However, in July 2020 the production site of the CMO was sold, and manufacturing activities were being stopped with immediate effect. Due to this situation, a different FP manufacturing site is licensed for blending, filling and freeze-drying of Nobivac DP Plus. Based on available batch data, the maximum bulk size is limited until production scale data from this manufacturer are available. In addition, a post approval change management protocol to license the manufacturing site for blending, filling and freeze-drying at production scale is provided. An increase to a production scale bulk size would then be implemented by variation (B.II.b.4.f) after the product is licensed.

Batch release for the lyophilisate fraction and solvent is performed by Intervet International B.V. in Boxmeer.

Manufacturing authorisations from the respective competent authorities are provided for all production sites.

All sites have been inspected. Certificates that detail the date of the last GMP inspection are provided. Therefore, additional inspections are regarded as unnecessary.

No GMP, GLP or GCP inspection is requested in connection with this application.

### *Overall conclusions on administrative particulars*

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the active substances and of the finished product manufacturing sites have been

satisfactorily established and are in line with legal requirements.

# Part 2 – Quality

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

Nobivac DP Plus is a lyophilised live vaccine without any adjuvant or preservative, containing canine distemper virus (CDV) strain Onderstepoort and canine parvovirus (CPV) strain 630a.
The CDV component is a conventionally attenuated live vaccine strain while the CPV component is a genetically modified hybrid CPV strain.
The solvent used with the vaccine has the same composition as the solvent for the live freeze-dried feline, canine and lapine vaccines of the Nobivac series.

## *Qualitative and quantitative particulars of the constituents*

### Qualitative and quantitative particulars

The vaccine is presented as lyophilisate in a single dose presentation with an accompanying solvent.

The active ingredients in the vaccine are live Canine distemper virus strain Onderstepoort and live, recombinant Canine parvovirus strain 630a.

The stabiliser contains hydrolysed gelatin, pancreatic digest of casein, sorbitol and disodium phosphate dihydrate.

The solvent is a sterile phosphate buffer, containing disodium phosphate dihydrate, potassium dihydrogen phosphate and water. The vaccine is mixed with the solvent prior to subcutaneous injection into dogs (puppies). The dose volume is 1 ml.

The qualitative and quantitative particulars of the vaccine suspension and the solvent are described adequately. No adjuvant or preservative are present in the vaccine.
All necessary certificates are provided.

## *Container and closure*

The product consists of two containers that should be mixed before use; one for the lyophilisate and one for the solvent.

The product is filled in hydrolytical class type I glass vials. The same vials are used for the freeze-dried vaccine and the solvent.
Vials are closed with rubber stoppers and aluminium caps.

The primary packaging materials for the product Nobivac DP Plus are considered compliant with the respective requirements in the Ph. Eur. and USP and their sterilisation is adequate.

## *Product development*

An explanation and justification for the composition and presentation of the vaccine has been provided.

Nobivac DP Plus is the successor product for the already licensed product Nobivac Puppy DP.
The distemper strain included is unchanged; the parvovirus strain however has been replaced with canine parvovirus strain 630a.

For the CDV component the batch of the vaccine used in the clinical studies had a titre of 5.1 $\log_{10}$ $TCID_{50}$/ds and this was therefore chosen as minimum titre.
The minimum titre of the parvovirus component is based on a dose response study with R&D material produced before the final production process was established; the respective study is provided.

The CDV component is produced in a conventional seed lot system using a master seed virus (MSV).

The production process of the CPV component starts with a transfection step with plasmid p630a instead of a seed lot.

Composition and volume of the solvent are the same as for the other live canine vaccines of the Nobivac range.

The formulation of most batches used during clinical studies is the same as that intended for marketing. Some of the studies were performed with single CPV antigen or CDV pre-MSV.

One batch of the CPV component used in a clinical study in cats differs in the production process from that described in the dossier. However, the applicant performed a comparative study of the consensus sequences of both products and no differences were found. The study can be considered valid. In the study, virus was shed from the cats, in summary it can be assumed that the virus used was sufficiently replication competent.

All excipients are well known, pharmaceutical ingredients and their quality is compliant with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC.

The CPV component is a live canine hybrid parvovirus strain named 630a based on the attenuated live vaccine strain 154 (backbone) and a capsid-coding insert derived from a currently circulating type 2c isolate.

The applicant describes the development of the recombinant CPV virus. The construction of the DNA plasmid and its use are described in detail. The plasmid is defined as a critical starting material. Information on the development process, the manufacturing process and the materials used during manufacture of the plasmid are presented. Furthermore, more details on the methods and specifications for testing are provided.

Satisfactory information on the strains used in the vaccine and development process of the plasmid are provided.

## *Description of the manufacturing method*

The production process is described in detail and relevant validation studies are provided.
A flow chart of the manufacturing process is provided.

The manufacturing process for both virus strains, their lyophilisation and the solvent are described in detail.

The production process for the CDV strain is a common production process for a viral vaccine employing a seed lot system and propagation of the virus in VERO cell culture.

The production process of the CPV strain is an innovative procedure using plasmid DNA and SAH-DK cells.

For the plasmid DNA, which is defined and characterised, it was demonstrated that passage 2 has the same sequence as the original plasmid.

Storage and stability of the plasmid are described and discussed. The storage period of the plasmid material is currently considered as unlimited.

A complete monograph on production and control of the plasmid is provided, which is considered acceptable. The DNA plasmid is defined as a critical starting material and a biological substance.

There is no in-process control test or finished product test performed which determines the amount of possible residual plasmid in the harvest and final product. An additional study was provided which demonstrated that no residual plasmid capable of transforming highly susceptible *E. coli* was present in the final product. Therefore, it is not necessary to implement a respective control test. Relevant validation studies on test methods are provided.
Data regarding the validation of viral titration of the CDV component is provided and the test for quantitative titration and identity is considered valid.

To produce the finished product, both vaccine viruses are combined with a stabiliser and M6/B8 medium, filled in glass vials and subsequently lyophilised.

Information on the lyophilisation process are described in the dossier.

Antibiotics are used in the manufacturing process. Their use was justified. A calculation on possible traces of antibiotics in the final product is provided and satisfactory. The amount of antibiotics possibly present in one dose of the final product is not considered as pharmacologically active.

## *Production and control of starting materials*

## Starting materials listed in pharmacopoeias

Certificates of analysis for all starting materials are provided and considered satisfactory.

Bovine serum is extensively tested by the supplier and in-house in accordance with Ph. Eur. monograph 2262 and the respective EU guideline EMEA/CVMP/743/00.

The quality and testing of the bovine serum are considered satisfactory.

## *Specific materials not listed in a pharmacopoeia*

## Starting materials of biological origin

CDV component

The description of the production and control of the MSV and working seed virus (WSV) for the CDV component is satisfactory. All relevant tests have been performed and the respective batch protocols are provided. The strain used in the product is identical to the one used in other products of the applicant.

The description of the production and control of the WSV for the CDV component is satisfactory.

Plasmid p630a

The information provided on the plasmid DNA as starting material for the CPV component is considered satisfactory.

The production process of the plasmid DNA, including information on the specific *E. coli* and other materials used for production and the complete manufacturing procedure have been provided. Information on all these starting materials including respective certificates and the description of the production process are also presented.

Detailed descriptions concerning the tests performed on the plasmid are provided.

VERO cells

Passage history and preparation at the company is satisfactory described.

Controls and tests carried out on the MCS are described and the respective quality control batch protocol for the MCS is provided.
The applicant provides a risk assessment and justification for omission of tests for several pathogens relevant for the target species (canine) and the species from which starting materials were obtained (porcine, bovine). These justifications are considered satisfactory. The respective documentation is provided.

Tests for identification of the species and karyology were performed; results on isoenzyme analysis, genetic stability and karyology are considered satisfactory.

In summary, the VERO cell line is considered suitable as production system for the CDV antigen component of Nobivac DP Plus.

SAH-DK cells

The SAH-DK cell line was developed by a company that has been acquired by the applicant.

Controls and tests were carried out on the MCS. The respective quality control batch protocol for the MCS is provided.
The applicant provides a risk assessment and justification for omission of tests for several pathogens relevant for the target species (canine) and the species from which starting materials were obtained (porcine, bovine). These justifications are considered satisfactory. The respective documentation is provided.

Tests for identification of the species and karyology were performed; results on isoenzyme analysis, species-specific immunofluorescence, genetic stability and karyology are considered satisfactory.

In summary, the SAH-DK cell line is considered suitable as production system for the CPV antigen component of Nobivac DP Plus.

The function, characteristics and origin of starting materials from animal origin is described. Satisfactory certificates of analysis for all listed substances are provided.

## Starting materials of non-biological origin

A commercially available plasmid buffer is used. It is a balanced salt solution free from components originating from humans or animals. A satisfactory certificate of analysis is provided.

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

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 satisfactory described.

## Specific measures concerning the prevention of the transmission of animal spongiform encephalopathies

A detailed TSE risk assessment is provided. A list of all starting materials of animal origin is provided indicating either that they all are derived from species which are not considered as TSE relevant, milk sourced from healthy animals in the same conditions as milk collected for human consumption, or a Certificate of Suitability was issued by the EDQM for this starting material.

Furthermore, Nobivac DP Plus is indicated for dogs, a species that is not naturally susceptible to TSE. It is concluded that the risk of TSE agents being transmitted by the use of this vaccine is negligible.

## *Control tests during the manufacturing process*

The detailed description of the in-process controls comprises viral titration and sterility testing of the CDV and CPV component. The tests performed are the same as those performed in final product testing.
Data from four independent production runs for CDV antigen and CPV antigen respectively are provided. Antigen batches were tested for virus titre and sterility. All batches were demonstrated to be sterile. The virus titres were demonstrated as above the proposed minimum titre in all batches.

## *Control tests on the finished product*

| **Parameter** | **Method** |
|---|---|
| **Solvent** | |
| Contents on average | Weighing |
| Appearance | Visual inspection |
| Colour | Examination of the degree of coloration in accordance with Ph. Eur. 2.2.2. |
| Clarity | Visual inspection in accordance with Ph. Eur. 2.2.1. |
| pH | Potentiometric method according to the Ph. Eur. 2.2.3. |
| Identity | Presence of sodium, potassium and phosphate according Ph. Eur. 2.3.1. |
| Sterility | According to Ph. Eur. 2.6.1. and Monograph 0062 |

| Parameter | Method |
|---|---|
| **Lyophilisate** | |
| Identity CDV | Cell culture method in accordance with Ph. Eur. monograph 0448 |
| Identity CPV | Cell culture method in accordance with Ph. Eur. monograph 0964 |
| Titre CDV (release titre) | Serial dilution on VERO cells and naphthalene black staining (CPE) |
| Titre CPV | Serial dilution on A72 cells and immunofluorescence staining |
| Sterility | According to Ph. Eur. 2.6.1. and Monograph 0062 |
| Test on Mycoplasma | According to Ph. Eur. 2.6.7. and Monograph 0062 |
| Extraneous agents | Cell culture test with monospecific sera against CDV and CPV |
| Residual humidity | Volumetric Karl Fischer titration |

The test methods used for the control of the finished product (identity, viral titre, sterility, absence of mycoplasma, extraneous agents testing, residual humidity) and the solvent (contents on average, appearance, colour, clarity, pH, identity, sterility) are briefly described and the specifications were provided.

Detailed description and validations of the tests are discussed; description and validation of all tests are considered satisfactory.

## Batch-to-batch consistency

Results for 4 batches of lyophilisate finally produced at the CMO and 3 batches of the solvent at production scale are provided. In addition, results of 12 pilot batches finally produced at the applicant's manufacturing site are provided.
All tests were performed. All results were within the specifications as proposed. Detailed batch protocols are provided.

## *Stability*

The following storage conditions and shelf life for the lyophilisate are approved:
After manufacture, the product may be stored for 24 months below -15 °C followed by the storage at +2 to +8 °C for up to 24 months. This results in a maximum storage period of up to 48 months, if the internal storage period is maximally extended.
The applicant provided the intended stability protocol and the already obtained stability data on four independent batches manufactured with aged antigen stored for different periods of time. All results provided remained within the proposed requirements.

Active substance

Stability data on antigen batches are provided, from which it can be concluded that a shelf life of one year for the CDV component and a shelf life of two years for the CPV component are justified.

Finished product

A statistical regression analysis for the stability data currently available is provided. It is concluded that the CPV titre is stable over the analysed time period.
For the CDV component a slight decrease in titre and for the residual moisture content a slight increase over the analysed time period was observed, this was taken into account for setting of the release parameters.

A shelf life of 24 months at +2 to +8 °C with a maximal pre-storage period of the final product in the applicant's storage facilities of 24 months at below -15 °C has been approved.

Stability at elevated temperatures

Data of samples of two batches of Nobivac DP Plus stored at +30 °C for 3 days, compared to samples stored at the proposed storage temperature of +2 to +8 °C were provided.
It was demonstrated that that the vaccine is stable for 3 days at +30 °C.
The SPC should therefore include the statement "Do not transport above 30 °C".

Photo-stability

Additionally, a photo-stability study was provided which showed satisfactory results. The transparent plastic packaging is suitable for its purpose. Stability of the product is guaranteed when kept in its original packaging under the recommended storage conditions.

Reconstituted product (in use-stability)

It was demonstrated that the vaccine is stable for at least 60 minutes after reconstitution with solvent.

Stability of the solvent

Data on three solvent batches stored for up to 48 months at room temperature and tested for filling volume, appearance, pH value and sterility are provided.
All parameters remained within the proposed specifications, except for one batch for which the pH value increased above the limit after 36 and 48 months.

A study to investigate the effect on solvent with an elevated pH was performed for 2 other products from the Nobivac range (Nobivac DHPPi and Nobivac Tricat Trio), having a similar composition as Nobivac DP Plus using 1 ml of solvent with a pH within the range and the maximum pH proposed during shelf life. Results show that the pH of the reconstituted product remained well within the normal range for parenteral products for at least 30 min after reconstitution when solvent with maximum pH was used.

As the solvent does not contain active ingredients, the storage conditions of the solvent in glass vials were defined as "No special precautions for storage".

## *Overall conclusions on quality*

Nobivac DP Plus is a lyophilised live vaccine without any adjuvant or preservative, containing as active substances live, attenuated canine distemper virus (CDV) strain Onderstepoort and live, attenuated canine parvovirus (CPV) strain 630a.

Maximum and minimum titre were proposed as follows:

CDV: 5.1 - 6.5 $\log_{10}$ $TCID_{50}$/dose

CPV: 5.1 – 6.7 $\log_{10}$ $TCID_{50}$/dose

The CDV component is a conventionally attenuated live vaccine strain while the CPV component is a genetically modified hybrid CPV strain. It is a recombinant vaccine virus derived from the backbone of the existing vaccine virus strain 154 and the attenuated capsid of a currently circulating type 2c isolate.

The CDV component is produced in a conventional seed lot system using MSV and WSV.

The CPV component production is started with a plasmid.

A scientific advice on this alternative approach was provided by the CVMP in 2017 (EMA/CVMP/SAWP/724500/2016). After additional information was provided, the applicant's approach is considered acceptable.

In the section product development, sufficient information on the origin of the strains is provided.

The qualitative and quantitative particulars of the vaccine lyophilisate and suspension, the solvent and the containers are described adequately. The necessary certificates are provided.
The composition of the solvent is the same as used for other canine and feline vaccines manufactured by the applicant.

The production process of vaccine and solvent is described in detail and relevant validation reports are provided.

The starting materials comply with the provisions of Ph. Eur. and the TSE risk assessment is adequate. The respective certificates of suitability are provided. The VERO cell line is considered as a suitable production system.
Information on the production of the plasmid 630a used as starting material for the CPV component is provided and adequate.

The in-process and finished product controls performed on Nobivac DP Plus are described in detail and appropriate specifications are proposed. The production process results in a reproducible composition of the vaccine.

Stability data were provided for storage of antigen bulk before filling, the finished product, stability at elevated temperatures, the in-use stability of the reconstituted product and the stability of the solvent. Additionally, a photo-stability study was provided.

Stability of the vaccine for 3 days at elevated temperature of 30 °C was demonstrated. The statement "Do not transport above 30 °C" is included in the SPC.

The shelf life for the final product with an internal storage of 24 months at below -15 °C followed by an external storage of 24 months at +2 to +8 °C was approved. The shelf life for the solvent is 48 months with no special precautions for storage.

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

The strategy followed to solve the situation created by the close of the CMO is acceptable.

# Part 3 – Safety

## *Introduction and general requirements*

Nobivac DP Plus (in the studies, reference is made to the name used during the development phase Nobivac Puppy DP Plus) is a live virus vaccine indicated for the immunisation of healthy puppies from four weeks of age against canine distemper and parvovirus infections presented in freeze-dried form in a vial and to be reconstituted with a solvent presented in a second vial. Nobivac DP Plus contains at least $10^{5.1}$ $TCID_{50}$, but not more than $10^{6.7}$ $TCID_{50}$ of CPV strain 630a and at least $10^{5.1}$ $TCID_{50}$, but not more than $10^{6.5}$ $TCID_{50}$ of CDV strain Onderstepoort per dose. No adjuvant or preservative is included.

The vaccine is the successor vaccine of the licensed product Nobivac Puppy DP, which contains live CDV strain Onderstepoort and live CPV strain 154 as active substances. Nobivac DP Plus contains the same live CDV strain Onderstepoort, but the live CPV vaccine strain 154 has been replaced by live CPV vaccine strain 630a (CPV630a).

Vaccine strain CPV630a is a hybrid virus that was derived from the backbone of the existing vaccine virus strain 154 and the attenuated capsid of a currently circulating type 2c isolate. The virus was constructed using molecular cloning techniques and is therefore termed a genetically modified organism (GMO), but does not contain any exogenous 'non-canine parvovirus' genetic material.

The hybrid vaccine strain CPV630a has the unique feature to achieve immunity in 4-week-old pups with high maternal antibody titres against CPV-2a, 2b and 2c.

One of the safety studies were performed with a 0.5 ml dose volume, but the 1 ml dose volume was finally chosen, as the end user is more familiar with it.

A full safety file in accordance with Article 12(3)(j) has been provided. Studies to determine the safety of the bivalent vaccine were performed in accordance with Ph. Eur. monographs 0964 ('*Canine parvovirosis vaccine [live]*'), 0448 ('*Canine distemper vaccine [live]*'), Ph. Eur. chapter 5.2.6 ('*Evaluation of safety of veterinary vaccines and immunosera*'), VICH GL 41, Directive 2001/82, Annex I, Title II as amended and EU Directive 2001/18/EC ('*Safety of genetically modified organisms*').

All reported laboratory studies were described to be GLP-compliant.

## *Safety documentation*

Four laboratory safety studies were conducted to investigate the safety of Nobivac DP Plus in 4-week-old puppies. Studies were performed to investigate the safety of the administration of a tenfold overdose and repeated dose of CPV630a, the effect of a tenfold overdose of CPV630a on thymus, the reversion to virulence of CPV630a and the dissemination of canine parvovirus strain 630a. A fifth laboratory safety study was conducted to investigate the reversion to virulence of the CDV strain Onderstepoort. This study was not in compliance with Ph. Eur. monograph 0448 requirements but is acceptable on the basis of the scientific advice provided.

One field study was performed in two kennels located in Morocco.

Additionally, five studies were conducted to investigate the safety of the CPV strain 630a in ferrets, safety of the administration of CPV strain 630a vaccine virus in cats and safety and spread of CPV strain 630a after oral administration in mice and in chickens.

The following studies were performed:

| ***Study title*** |
|---|
| Safety of 10x overdose followed by repeated dose |
| Effect on the thymus of an overdose of canine parvovirus strain 630a |
| CPV reversion to virulence |
| CDV reversion to virulence |
| Dissemination of the CPV strain 630 |
| Disinfection of CPV 630a contaminated animals' facilities using formaldehyde fumigation |
| Safety of CPV strain 630a in ferrets |
| Safety and spread of CPV strain 630a in mice after oral administration |
| Safety and spread of CPV strain 630A after oral administration to the non-target species chicken |
| Safety of the administration of CPV strain 630a vaccine virus in cats |
| Study to Investigate the Dissemination of Canine Parvovirus strain 630a in Ferrets early after Inoculation |
| Field study to assess the safety and efficacy of Nobivac DP Plus |

## *Laboratory tests*

## *Safety of the administration of one dose and an overdose*

Studies applicable to live vaccines include the investigation of the safety of a single dose. However, this study was not performed as an isolated study. The safety of a single dose was assessed in the frame of the assessment of the safety of the repeated administration of a single dose after administration of a tenfold overdose.

As the basic application for this vaccine consists of the administration of one single dose to puppies from 4 weeks of age onwards, and in order to avoid animal use for an additional safety study as well as in conformity with the principles of Directive 2010/63/EU, the CVMP accepted this approach.

The study was conducted to examine the safety of a tenfold overdose of the bivalent test vaccine administered subcutaneously to 4-week-old specific pathogen free (SPF) Beagle puppies followed by a single repeat dose administration after 3 weeks. Examinations of possible interactions with Nobivac L4 by mixed vaccination and Nobivac KC by intranasal administration were included.

Ten naïve 4-week-old Beagle pups of mixed sex were assigned to two groups of 5 animals each:

- Group 1: 5 naïve pups were subcutaneously vaccinated with a tenfold overdose on day 0 (D0), i.e. CDV $10^{7.5}$ CPV $10^{7.7}$ $TCID_{50}$/ds of Nobivac DP Plus mixed with Nobivac L4. Pups additionally received Nobivac KC via the intranasal route.
- Group 2: 5 naïve pups were subcutaneously vaccinated with a tenfold overdose on D0, i.e. CDV $10^{7.6}$ CPV $10^{7.8}$ $TCID_{50}$/ds of Nobivac DP Plus reconstituted with the Nobivac Solvent. Pups additionally received Nobivac KC via the intranasal route.

Three weeks after the first vaccination (D21), a single dose was applied to the same puppies via the subcutaneous route:

- Group 1: Nobivac DP Plus CDV $10^{6.5}$ CPV $10^{6.7}$ $TCID_{50}$/ds reconstituted in a single dose of Nobivac L4.
- Group 2: Nobivac DP Plus CDV $10^{6.5}$ CPV $10^{6.7}$ $TCID_{50}$/ds reconstituted with the Nobivac Solvent.

For treatment of a mild respiratory infection observed in two pups of group 2, antibiotics were given for five days to all ten pups from D21 to D25. The applicant stated that the two pups became most likely infected with *Bordetella (B.) bronchiseptica* during the transport from the colony to the laboratory facilities.

Following the administration of the overdose vaccination, a soft diffuse thickening (discrete non measurable swelling) was observed at the injection site in one pup of each group. Following the single repeat dose vaccination, a majority of the vaccinated pups showed a similar soft diffuse thickening. Local reactions disappeared within 2 to 6 days after vaccination. No abnormal clinical observations were recorded, and no clinical observation data were observed, except for the two pups suffering from mild respiratory signs. The weights of all animals increased continuously throughout the study and there was no increase in body temperature in any pup following either overdose or repeat single dose vaccination.

Following the overdose vaccination, all five pups of group 1 which had received Nobivac DP Plus mixed with Nobivac L4 showed a drop in white blood cells (WBC) below 50% of their mean baseline by D3, 5 and 7, which returned to normal by D10 but dropped again by D16 to 70–80% of the mean baseline. The value of the circulating white blood cells for the pups of group 2 remained relatively close to the baseline mean by D7, whereas an increase of more than 100% was seen by D10. This leucocytosis was probably due to a *B. bronchiseptica* infection during the transport as described above.

This study meets the requirements of Ph. Eur. monographs 0964 and 0448, Ph. Eur. chapter 5.2.6 and Directive 2001/82/EC.

Haematological results indicate that the test vaccine alone does not cause a significant reduction in the number of circulating white blood cells for a longer time when given as an overdose, whereas the mixed use of the test vaccine with Nobivac L4 may lead to a decrease of white blood cells after vaccination. Leucodepletion following mixed vaccination of 4-week-old puppies with Nobivac L4 may increase the susceptibility of 4-week-old pups to other infectious agents during 7 days after vaccination or even longer after administration of an overdose (i.e. D16).

It can be concluded that the administration of one overdose of Nobivac DP Plus containing the tenfold of the maximum potency of antigens by the recommended route was found to be safe for puppies of 4 weeks of age. The administration of one dose to the same puppies 3 weeks later was found to be similarly safe.

However, as the mixed use of Nobivac DP Plus and Nobivac L4 is not supported by the provided data, the advice regarding the mixed use with Nobivac L4 was waived.

### *Safety of one administration of an overdose*

The study was carried out to determine the effect of an overdose of canine parvovirus strain 630a on the thymus of pups approximately 4 weeks of age. Here, pups received the monovalent CPV630a vaccine strain.

In this overdose study, eight naïve less-than-4-week-old Beagle pups of mixed sex were assigned to two groups of 4 animals each. Pups of group 1 were subcutaneously vaccinated (CPV $10^{8.0}$ $TCID_{50}$/ds)

and pups of group 2 represented non-vaccinated controls. On D14 and D21, two dogs from each group were euthanised to obtain a panel of sixteen different organs and tissues including thymus for histological examination, as required by Ph. Eur. monograph 0964, and virus isolation. White blood cell counts were determined from blood samples collected at regular intervals. Serology was done with serum samples collected on D0 and D14.

Because of a breakdown in biosecurity that occurred during the initial study period as indicated by detection of seroconversion in the non-vaccinated pups of group 2 on D14, an additional three 6–7-week-old pups served as replacement animals. The report of the results of group 2 and replacement pups is therefore divided into two parts: results from part 1 before biosecurity had been compromised and results from part 2 describing the results and actions taken thereafter. The three additional pups replaced the non-vaccinated controls of group 2.

All pups remained healthy. No abnormal clinical observations and no adverse effects were recorded. The weights of all animals increased continuously throughout the study, supporting that all dogs developed and grew normally. The rectal temperatures of all animals remained within the physiological range for the entire observation period. Following the administration of the overdose, no adverse effects were recorded at the injection site.

By D14 post vaccination, all group 1 pups had seroconverted, with hemagglutination inhibition (HAI) antibody values up to 1:8192. As mentioned above, all group 2 pups had similarly seroconverted.

Haematological results did not show any evidence for a drop of white blood cells counts towards the 50% baseline.

Comparative histological examination of the thymus from pups of group 1 and the replacement group did not show evidence for thymic atrophy on D14 and D21 in the vaccinated pups. However, minimal to slight histopathological changes in the thymus were observed in three out of four of the unintentionally infected control animals, both on D14 and D21. The day on which the pups became infected due to the biocontainment breakdown and the infection titre was not known and therefore conclusions regarding the histopathological changes cannot be drawn. The histopathological description of the lesions was provided. In the replacement pups, CPV630a virus could neither be detected in serum samples nor in 10% (w/v) thymus and tonsil homogenates, whereas tissue homogenates from pups of group 2 were CPV-positive as demonstrated by virus isolation. Genomic sequence determination confirmed unique 630a sequences, thereby also confirming that group 2 had been infected with CPV vaccine strain from the room housing the group 1 pups.

As, after administration of an overdose of CPV strain 630a, there were no adverse histological observations made on the thymus tissue on D14 and 21 post inoculation or any other adverse clinical observations in pups of approximately four weeks of age, the requirement of Ph. Eur. monograph 0964, section 2.3.1.2 is met.

The guidance on component-specific safety requirements of Ph. Eur. monograph 0964 (‘*Canine parvovirosis vaccine [live]*’) was considered. The study is considered valid even though the control pups had to be replaced and only three pups were used instead of the required four control animals. After administration of an overdose of canine parvovirus strain 630a, no adverse histological changes on the thymus tissue of approximately 4 weeks aged pups were observed.

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

For clinical safety and injection site safety after the repeated administration of one dose, reference is made to the above 10x overdose and repeated dose safety study.

### *Examination of reproductive performance*

The vaccine is not intended for pregnant animals. Therefore, studies in pregnant animals were not performed. The advice '*The safety of the veterinary medicinal product has not been established during pregnancy*' is included in the SPC Section 4.7 and in the PL section 12.

### *Examination of immunological functions*

Immunological functions should be examined where the product might be expected to adversely affect the immune response of the vaccinated animal. Mixed vaccination of puppies with a leptospirosis vaccine did not result in a negative effect on the serology of the bivalent vaccine Nobivac DP Plus. Reference is made to the results of the field trial. The applicant concluded that the product Nobivac DP Plus does not adversely affect the immunological functions.

In the overdose study in pups receiving mixed vaccination, a transient drop of the white blood cells below 50% of their mean value was noted followed by another drop. In the non-target species cat, a similar drop of white blood cells towards the 50% baseline was similarly noted.

### *Special requirements for live vaccines*

To fulfil the special requirements for live vaccines according to Directive 2001/82/EC as amended, Title II Part 3.B.6 and Ph. Eur. monographs 0448 and 0964, several laboratory studies in the target species were carried out.

## Spread of the vaccine strain

Virus shedding of CPV 630a or CDV strain Onderstepoort after vaccination were closely monitored in two respective reversion to virulence studies.

## Dissemination in the vaccinated animal

Dissemination in the vaccinated animal is required for live GMO strains (Section 3.2.1.2 of EMEA/CVMP/004/04-FINAL). Therefore, a dissemination study was only carried out for canine parvovirus strain 630a.

One dissemination study was carried out to investigate the *in vivo* dissemination and duration of virus shedding following administration of CPV strain 630a to six 4-week-old SPF Beagle pups and one non-vaccinated control pup.

The animals were assigned to three groups. Pups from groups 1 and 2 with three animals each were subcutaneously vaccinated with a high dose ($10^{7.0}$ $TCID_{50}$/ds) and euthanised on D4 (group 1) and D11 (group 2). Group 3 comprised a single non-vaccinated/non-in-contact control pup that served as a donor for control tissues, blood and faeces samples on D4.

A panel of tissue samples comprising sixteen different organs was collected and homogenized (10% [w/v] in PBS) to examine dissemination of CPV630a in the body of vaccinated pups including important viral target organs (e.g. thymus, intestine, myocardium) and skin around the injection site. Blood was collected on D0, D4 and D11 for serology and only for group 2 daily for virus isolation. Rectal swabs were taken daily to determine viral shedding. In addition to serology in serum, possible presence of CPV630a-specific antibodies in tissue homogenates was examined by HAI.

The validity of the virus detection methods, i.e. possible inhibitory matrix effects, was shown by using replica homogenates from vaccinated and non-vaccinated pups spiked with $10^{5.0}$ $TCID_{50}$/ml CPV 630a virus.

Cell culture-based virus isolation and PCR were applied to examine CPV630a dissemination in the body of the pups. However, technical inconsistencies with the PCR method were encountered, and the inconclusive PCR results were not reported.

Tissue samples from the myocardium were taken for histological examination of myocarditis by the usual histopathological method.

No abnormal clinical observations were recorded. All pups remained healthy and no adverse effects were recorded after vaccination during the course of this study. The scoring protocols used were provided by the applicant. The body weights were not presented in this report, but they were recorded daily. Rectal temperatures of all animals remained within the physiological range for the entire observation period with no indication of pyrexia.

All vaccinated pups seroconverted successfully showing positive titres from D4 onwards and reaching levels of 8192 HAI units on D11 in group 2. Low CPV630a-specific HAI titres (16–64) could be detected in tissues on D4, and levels increased thereafter. On D11, the highest antibody values were in the thymus (512–1024 HAI units) and slightly lower values in all the other tissues (64–256 HAI units). The unvaccinated pup remained seronegative throughout the observation period.

Rectal swab supernatant titration revealed virus shedding appearing in group 1 on D+2 and continued until D+4 with considerable shedding (titre of > 6.64 $\log_{10}$ $TCID_{50}$/ml). In group 2, shedding was seen for one pup on D1 (2.45 $\log_{10}$ $TCID_{50}$/ml), on D2 for another pup (2.80 $\log_{10}$ $TCID_{50}$/ml), on D3 for all three pups of this group with different titres (2.45, > 6.64 and 5.24 $\log_{10}$ $TCID_{50}$/ml, respectively), continuing on D4 and 5 for all pups until D6 for two of the three pups (no virus, 3.84 and 3.49 $\log_{10}$ $TCID_{50}$/ml, respectively). There was no virus shedding detected in the non-vaccinated pup.

In the tissue samples of thymus, spleen, intestinal tissues, lymph tissues, tonsil, liver, and injection site skin CPV630a virus was detected in group 1 pups on D4. Here, the thymus had the greatest amount of virus (> 6.64 $\log_{10}$ $TCID_{50}$/ml), typically 100x higher than any of the other tissues (smallest amount injection site: mean 3.03 $\log_{10}$ $TCID_{50}$/ml, highest amount tonsil: mean 5.59 $\log_{10}$ $TCID_{50}$/ml, most tissues: mean 4.20 $\log_{10}$ $TCID_{50}$/ml). Very little or no virus was detected in the kidney, lung, brain tissues and heart. Different to group 1, there was no CPV 630a isolated from any of the tissues taken from the group 2 pups. This decrease of disseminated virus is most probably a result of virus neutralization by the high levels of CPV antibodies produced following vaccination.

Histological examination of heart myocardium samples showed no macroscopic or microscopic abnormalities or evidence of inflammatory processes in any of the pups.

There was no evidence of any adverse effects following administration of CPV630a in pups of approximately four weeks of age. CPV630a was shown to have disseminated to various tissues within four days of administration. However, virus could not be detected in the same tissue types eleven days post administration due to neutralizing levels of antibodies.

The study is valid. The fact that the virus-spiked tissue homogenates were only found positive in the tissue samples from group 1, exhibiting low CPV630a-specific antibodies, whereas in group 2, all virus-spiked tissue homogenates with relatively high HAI titres were virus-negative, was described in detail in the safety in ferrets study. Similarly, these data indicated that virus could not be isolated in the presence of neutralising antibody titres, or in the occasional presence of tissue toxicity. Any virus, if present, was assumed to be either neutralised or otherwise inactivated and unable to replicate. Unfortunately, PCR-based virus detection results could not be used to verify this assumption.

Consequently, it is not possible to definitively state that vaccine virus was entirely absent in homogenates which yielded a negative result.

## Reversion to virulence of attenuated vaccines

CDV vaccine strain

The reversion to virulence of the CDV vaccine strain was investigated in one study using the original CDV MSV. Three six-week-old pups were inoculated intramuscularly, subcutaneously and intranasally (3x 1 ml of $10^{6.92}$ $TCID_{50}$/ml) with a total of $10^{7.4}$ $TCID_{50}$. One dog was not inoculated and served as in-contact control. Nasopharyngeal swabs were taken daily, starting on D0 through D21. Isolation and titration were done using VERO cells. No virus could be isolated from any dog. Therefore, individual nasopharyngeal swab material from the previous passage from D3 through D8 were used to inoculate two new pups. These two dogs were observed as stated before and nasopharyngeal swabs were then passaged to another two pups. Since no virus could be isolated from the third passage, the study was terminated at this passage level. Seroconversion at passage 1 confirmed the presence of CDV infection. The in-contact control dog remained seronegative. The lack of seroconversion at passage levels 2 and 3 and the lack of virus shedding indicate that the virus failed to be transmitted. All dogs at passage levels 2 and 3 remained seronegative. The non-vaccinated in-contact pup of the first group remained virus and seronegative. None of the pups developed clinical signs nor pyrexia or a disturbed body weight gain.

The inoculation of the first backpassage group stimulated a good antibody response. The CDV MSV could not be isolated at any passage nor did it spread to the in-contact dog. All pups remained clinically healthy. No reversion to virulence of the CDV after being backpassaged through dogs three times could be demonstrated.

This study is not fully compliant with the current Ph. Eur. monograph 0448, but reference is made to a scientific advice (EMA/CVMP/SAWP/724500/2016). The CVMP concluded that a new reversion to virulence study is not required if the new master seed is produced within the maximum allowed number of passages (new MSV+4). The applicant followed this advice. The new MSV corresponds to old MSV+1.

The history of the long-term use of the vaccine strain combined with the original study results were suitable to show that the CDV MSV does not present a risk of reversion to virulence.

CPV630a vaccine strain

The reversion to virulence of strain CPV630a was investigated in one study taking into account the requirements of Ph. Eur. chapter 5.2.6 and Ph. Eur. monograph 0964, but the requirements as given are met only partially. The monograph 0964 states that WBC counts and histological examination of the thymus should be considered in the evaluation. However, these data were not collected. Instead the sequence of the $5^{th}$ passage was compared with the consensus sequence of canine parvovirus strain 630a.

Eighteen SPF Beagle pups were assigned to 5 groups. Groups 2 and 3 consisted of two pups, groups 1 and 4 comprised three pups and eight pups were included in group 5. Group 1 pups subcutaneously received an overdose (CPV $10^{7.5}$ $TCID_{50}$/ds) and pools of faecal collections (D2 to D6) were then oronasally transmitted from group to group thereafter.

All pups remained healthy. No abnormal clinical observations and no adverse effects were recorded after vaccination or inoculation of the test material during the course of this study. There were no signs of diarrhoea or lethargy in any of the dogs throughout the study. The weights of all pups increased continuously throughout the study. Rectal temperatures of all animals remained within the physiological

range for the entire observation period with no indication of pyrexia with only one exception on a single day.

In group 1, virus shedding via faeces shedding began on D2, peak shedding was seen on D3 and D4. There was no shedding detected from D5 onwards. Virus was then shown to be shed following administration of faecal samples via the oronasal route. Virus shedding appeared to begin two days later on D4 and continued for a longer time with considerable shedding detected on D4, D5 and D6 in group 2. In group 3, shedding was seen from D3 up to D6. In group 4, virus shedding appeared to begin on D2 and continued for a longer time, with considerable shedding detected on D3 until D7. In group 5, shedding was seen from D3 and continued for two days up to D5 for two pups and up to D6 for the two other pups.

Titration of the faecal pool materials that were prepared from faecal collections from D2 until D6 showed the following mean titres of faecal pools 1, 2, 3 and 4 administered to the pups (second, third, fourth and fifths passage): 1st faecal pool < 2.3 $\log_{10}$ $TCID_{50}$/g, 2nd faecal pool 6.2 $\log_{10}$ $TCID_{50}$/g, 3rd faecal pool 6.6 $\log_{10}$ $TCID_{50}$/g and 4th faecal pool 6.3 $\log_{10}$ $TCID_{50}$/g. The fact that the amount of virus in the first faecal pool (administered to group 2) was much lower than that determined for the second, third and fourth faecal pools was explained by the lower amount of shedding in group 1 pups, where shedding had effectively stopped by D5.

After five sequentially successful passages of CPV630a through five groups of young pups, there was no evidence of reversion to virulence.

The study demonstrated that strain 630a can be spread via faeces of recently vaccinated dogs.

All pups developed and grew normally, and the rectal temperatures of all animals remained within the physiological range for the entire observation period. By appropriate sequencing data submitted by the applicant, it could be shown that there was no difference between the vaccine virus (inoculum) and virus recovered from faecal samples of the virulence study. In conclusion, even though the study design did not fulfil all general and specific requirements of the Ph. Eur., it is acknowledged that the reversion to virulence study did not indicate any genetic (sequencing results after 5 passages show that the sequence was identical to the consensus CPV 630a sequence) or clinical signs indicating reversion to virulence of vaccine strain CPV630a in pups after the 5th passage.

## Biological properties of the vaccine strain

The CPV component is a live canine hybrid parvovirus strain named 630a based on the attenuated live vaccine strain 154 (backbone) and a capsid-coding insert derived from a currently circulating type 2c isolate. It induces protection against canine parvovirus within 3 days in naïve animals and it has the unique feature of achieving immunity in pups with levels of maternal antibodies in the highest range.

The CDV component is a well-known vaccine strain. No special biological properties are known for the vaccine strain.

## Recombination or genomic reassortment of the strains

Canine distemper virus is a paramyxovirus. Being a single-stranded RNA virus, it is not readily amenable to recombination.

Canine parvovirus is a single-stranded DNA virus that replicates in the nucleus of the host cell. It is therefore evident that recombination can occur when two different canine parvovirus strains are present in the same cell. Therefore, opportunities for recombination between vaccine virus and field strains of

canine parvovirus do exist. This event is just as natural as a recombination between two field strains and it is not to be expected that a recombinant virus will have virulence factors, which are not already present in the contributing parent field strain.

Each of the two viruses concerned has a single genome. Therefore, genomic reassortment will not occur.

## *User safety*

A user risk assessment in accordance with applicable guidelines has been provided. The following elements are discussed by the applicant: hazard identification, exposure assessment, assessment of the consequence of a hazard occurring, risk characterisation and risk management and communication. Accidental self-injection during preparation or administration of the vaccine was identified as the main exposure scenario. As the active ingredients are live attenuated antigens, which are not considered zoonotic and the excipients are not regarded as hazardous, the risk for adverse effects in relation to an accidental self-injection is considered to be low. Furthermore, the person who will administer the vaccine is a trained professional, which reduces the risk for exposure via accidental self-injection.

The CVMP acknowledged the user risk assessment, which is considered satisfactory. It addresses the exposure situations under normal conditions of use and in case of foreseeable accidents, and this is reflected in the SPC and product literature.

## *Study of residues*

Performing a study of residues is not applicable, as Nobivac DP Plus is not intended for food producing species.

## *Interactions*

Under *Interactions*, the applicant proposes additional information concerning interactions with two other Nobivac products based on two safety studies (10x and repeat dose laboratory study, field trial), in which the applicant wanted to demonstrate that Nobivac DP Plus can be given in combination with Nobivac L4 instead of solvent and that Nobivac DP Plus can be concurrently administered with Nobivac KC.

Nobivac L4 is a centrally authorised inactivated vaccine for active immunisation of dogs against four different *Leptospira* serogroups. The minimum age for vaccination is 6 weeks.

Regarding the 10x overdose and repeat study, the applicant concluded that the clinical observations remained within the limits as stated in SPC section 4.6 of Nobivac L4. However, all five pups that received Nobivac Puppy DP Plus mixed with Nobivac L4 showed a transient drop of white blood cells counts below 50% of their mean baseline value, whereas all 5 pups vaccinated with Nobivac Puppy DP Plus reconstituted in solvent did not. The mixed use of Nobivac DP Plus and Nobivac L4 is not supported and the advice regarding the mixed use with Nobivac L4 was waived.

With regard to the field trial, stronger adverse reactions were observed when the lyophilisate was dissolved in Nobivac L4 compared to the reactions seen in the 10x overdose and repeat study using the vaccine in question dissolved as provided in the SPC (lyophilisate and solvent).

Nobivac KC (also named Nobivac BbPi) is a lyophilised vaccine containing live *B. bronchiseptica* and live canine parainfluenza virus (CPiV) as active ingredients, indicated to reduce clinical signs induced by *B.*

*bronchiseptica* and CPiV and to reduce shedding of CPiV for periods of increased risk. The vaccine is administered intranasally to dogs from 3 weeks of age onwards.

In the laboratory 10x overdose and repeat safety study, Nobivac Puppy DP Plus was concurrently administered with Nobivac KC. The applicant concluded that the clinical observations remained within the limits as stated in section 4.6 of the Nobivac KC SPC.

However, to reflect the absence of sufficient data in support of efficacy for Nobivac KC when used in combination with Nobivac DP Plus, the following statement is included in section 4.8 of the SPC.

**Interaction with other medicinal products and other forms of interaction**

Safety data are available which demonstrate that this vaccine can be administered on the same day but not mixed with a vaccine of the Nobivac series containing *Bordetella bronchiseptica* and canine parainfluenza virus components for intranasal administration. Efficacy after concurrent use has not been tested. Therefore, while safety of concurrent use has been demonstrated, the veterinarian should consider this when deciding to administer the products at the same time.

## *Field studies*

One field study was conducted in two private kennels located in Morocco to evaluate safety and efficacy of Nobivac DP Plus. The vaccine was reconstituted in a low volume (0.5 mL) of Nobivac L4 and administered subcutaneously. Two different Nobivac DP Plus batches were used.

The study comprised 89 pups of different breeds. From one kennel, only one litter with three pups was included in the study. The other pups were located in the second kennel. In one kennel, there were 3 cases of CPV infection in 2015 and in the other kennel there were an unknown number of kennel cough cases in 2016, but no new cases since then were noticed regarding both diseases. All 89 pups were vaccinated, and no control group was included. The pups included in the field study were on average 5 weeks of age (34.4 ± 5.2 days), with a range between 25 and 43 days.

One litter with three puppies was excluded prior to the vaccination, since 2 out of 3 puppies developed clinical signs of illness and died thereafter. From the remaining 89 puppies, several puppies suffered from gastrointestinal (*E. coli* and rotavirus infections, severe ascariasis) or sarcoptic mange infection. Ocular/nasal discharges occurred in a number of puppies. Two puppies died during the study. The applicant stated that these diseases were probably not related to vaccination and submitted information on how the diarrhoeic pups (13 in total) were diagnosed with specific conditions such as diarrhoea due to change of feed, rotavirus or *E. coli* infection. Body weights were recorded to determine the blood volume that could be withdrawn, but not evaluated as a clinical parameter. Rectal temperatures were recorded between D-3 and D4 but not thereafter. No clinically relevant changes in rectal temperature were noticed.

During or immediately after vaccination, 17% (15 out of 89) of the puppies showed whining or scratching at the injection site (whining: 9 puppies, scratching: 3 puppies, pain: 2 puppies, whining and scratching: 1 puppy). These reactions were noted immediately after or during vaccination. In all cases, immediate reactions resolved quickly thereafter (within approximately one minute).

Forty-nine per cent (44/89) of the puppies showed a local transient reaction at the injection site, which resolved between 5 (66%) and 14 days (98%) after appearance, with one exception. An estimated maximum measured size of swelling up to approximately 3 cm (2 puppies) was observed but the majority of the puppies (61%) showed a maximum swelling of ≤ 1 cm. Both hard and soft swellings were scored, and in 15 puppies the reaction was scored as painful and in 6 puppies as painful and/or warm.

17% (15/89) had an abnormal clinical score (score 1) for general health after vaccination. 14 puppies came from three litters and were scored as 'less active, minor signs of discomfort' for several hours to two days. In addition, 8% of the puppies (7/89) from these litters showed a reduced feed intake for one day after vaccination. Six of them were also scored as less active for general health. The most common reaction 'less active' occurred in eight cases within four hours after vaccination. Those puppies came all from the same litter and this reaction was considered to be a reaction to the animal handling. Occurrence of reduced activity is listed in the SPC.

The overall health status in both kennels was characterised by a relatively high morbidity rate by non-vaccine related diseases. Nevertheless, this was not considered to be influencing the results of the study as the housing and daily husbandry of the bitches with pups were comparable to the EU. Regarding the surveillance of the two breeders by a veterinary medical officer, a statement and additional information are provided by the applicant, which confirm that surveillance in Morocco is comparable to the situation in the EU.

The study did not include a control group (either non-vaccinated or vaccinated with a comparable vaccine), which was explained satisfactorily by the applicant (see efficacy part). Clinical parameters such as the body weight evolution were not reported or were only reported during a very short period of time, not covering the whole observation period. Due to the study design it is not possible to draw any conclusion regarding an association between weight gain retardation and vaccination with Nobivac DP Plus from the field data alone.

The mixed use of Nobivac DP Plus and Nobivac L4 is not supported; the advice regarding the mixed use with Nobivac L4 is waived.

## *Environmental risk assessment*

The CPV strain 630a has the same attenuating backbone and capsid amino acid changes as canine parvovirus strain 154, which has a history of safe use for more than 25 years. There are no indications that recombination or reversion to virulence occurs under field conditions. Therefore, the risk that a hazard occurs is considered as low.

For the CDV component, there are no potential risks for the environment since this strain is not shed by vaccinated animals. The CDV strain is derived from the same seed lineage as the CDV that has been used for a long period by the company as a live vaccine strain. Environmental problems and reversion to virulence have not been reported for CDV derived from this lineage.

The CPV strain is intended for pups from 4 to 6 weeks of age and, at this age, the animals remain within the home or kennel and have not yet been taken for a walk. This reduces the chance that wild animals or other non-vaccinated domestic dogs will come into contact with the vaccine strain. If other animals would unintentionally or intentionally (ferrets or cats) come into contact with the vaccine strain, it is unlikely that a hazard would occur since it has been demonstrated that canine parvovirus strain 630a is safe for non-target species which may come into contact with the vaccine. Therefore, the risk that a hazard would occur is considered as negligible.

Taking all the risk factors in consideration, the assessment of the level of risk for Nobivac DP Plus can be considered as being low. This is also reflected in the product literature. Phase II environmental risk assessment is not considered necessary. Nevertheless, a warning is included in section 4.5 in the SPC regarding the possibility of cats becoming infected albeit without any signs of disease.

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

Nobivac DP Plus or rather the active substance of this vaccine, CPV strain 630a, falls within the scope of Directive 2001/18/EC on the deliberate release into the environment of genetically modified organisms.

Detailed information on the parental virus CPV strain 154 has been provided, including safety studies of the parental strain even if not required by guidelines or the Directive.

Detailed information on the possible risks for humans and for the environment has been provided.

The vaccine is compliant with Directive 2001/18/EC.

Nobivac DP Plus does not infect humans and is restricted to the infection of dogs and other animals of the family Canidae, as well as members of the family Felidae (e.g. domestic cats) and Procyonidea (e.g. raccoons).

The vaccine strain was generated by a multistep process using molecular cloning techniques. The vaccine virus does not contain any non-parvovirus sequences, which were exchanged as described above. Reversion to virulence studies (*in vivo*) did not show any tendency for reversion.

The vaccine virus was shed from vaccinated animals via faeces for a limited time span of 8 days. In non-target species studies, cats inoculated with CPV strain 630a shed the virus via faeces within the first week post-inoculation and the vaccine virus did also spread to in-contact animals. These animals also showed faecal shedding without any clinical signs or loss of body weight. A mild transient drop in white blood cell counts in inoculated cats was observed, which coincided with faecal shedding. Inoculated and in-contact cats did not develop any signs of infection but CPV-specific antibodies. Similarly, ferrets inoculated with CPV strain 630a did not show any clinical signs and developed antibodies against CPV. Ferrets did not shed the virus via faeces and virus could not be isolated from the tissues after infection. Another study in ferrets investigated earlier time points. Results showed that the virus was not shed via faeces and ferrets did not show any clinical signs. Virus could not be isolated from tissue samples. Studies in mice and chickens inoculated with the CPV vaccine strain 630a did not lead to shedding, spreading or any clinical signs, but inoculated animals showed a CPV-specific seroconversion. A warning is included in the SPC indicating that the vaccine virus may spread to cats and shed to other cats after contact with primary vaccinated dogs or faeces of dogs without the induction of clinical signs.

Taken together, any risk emerging from the use of the attenuated vaccine viruses is expected to be negligible for humans and low for the environment.

## *Overall conclusions on the safety documentation*

The safety of Nobivac DP Plus was investigated in laboratory studies and one field trial. All laboratory studies were carried out with the most sensitive category of target animals, i.e. 4-week-old SPF pups. In the laboratory studies, batches containing the maximum release titre were used. In the field trial, standard batches were used. The vaccine was administered by the subcutaneous route, as recommended. Laboratory studies were reported to be compliant with GLP principles.

In the tenfold overdose study, the test vaccine was either given alone or administered in a mixed vaccine together with Nobivac L4. Concurrently, one dose of Nobivac KC was administered intranasally. Three weeks later, a single dose of Nobivac DP Plus was given alone or mixed with Nobivac L4. As the basic vaccination for this vaccine consists of the administration of one single dose (1 ml), and in order to avoid animal use for an additional safety study, this approach is acceptable. Here, the test vaccine is considered safe for the administration of one dose and the tenfold overdose. Local reactions were

adequately mentioned in the SPC. Regarding white blood cells, the test vaccine alone has no negative effect, but the mixed use of Nobivac DP PLUS together with Nobivac L4 may lead to a decrease of white blood cells after vaccination.

In the second laboratory study, a tenfold overdose of a monovalent CPV 630a vaccine was administered. In this study, the effect of an overdose of CPV strain 630a on the thymus in comparison to non-vaccinated control animals was investigated. No histopathological changes were observed in any of the organs.

In the field trial, the vaccination of Nobivac DP Plus mixed with Nobivac L4 induced stronger local adverse reactions, with 17% of the puppies having an abnormal clinical score (score 1) for general health after vaccination and up to 8% of the puppies showing a reduced food intake for one day after vaccination.

The examination of immunological functions was conducted based on the estimation of serological titres after vaccination. Mixed vaccination of puppies with a leptospirosis vaccine did not result in a negative effect on the serology of this product.

A reversion to virulence study of the CPV 630a vaccine was carried out by passaging the CPV630a strain sequentially through five groups of pups. The vaccine virus strain was shown to be genetically and phenotypically stable.

The vaccine virus spreading via faeces of recently vaccinated dogs was shown. Following administration of faecal samples containing the vaccine, virus was also shed after each passage.

A dissemination study using virus isolation based on cell culture demonstrated that CPV 630a was shed by vaccinated pups from D1 to D2 onwards and could be detected in a number of organs and tissues with the highest levels found in the thymus. The detection method failed to detect virus as soon as CPV-specific antibodies reached a certain level indicating that immune-complexed CPV could not be detected.

A reversion to virulence study of the CDV component was carried out demonstrating that the CDV MSV does not present a risk of reversion to virulence.

A user safety risk assessment was made in accordance with the '*Guideline on user safety for immunological veterinary medicinal products*'. No hazard was identified, and the risk can be considered very low. For the risk of self-administration, the standard warning was included in the product literature, which is acceptable.

No study of residues was performed, as Nobivac DP Plus is not intended for food producing species.

A laboratory 10x overdose and repeat safety study and a field trial were performed, in which the applicant wanted to demonstrate that Nobivac DP Plus can be mixed with Nobivac L4 instead of solvent and that Nobivac DP Plus can be concurrently given with Nobivac KC. The impact on the WBC was reflected in the proposed SPC. However, the proposed mixed used of Nobivac DP Plus with Nobivac L4 is not supported and therefore the advice regarding the mixed use with Nobivac L4 is waived.

A recombination event between the vaccine CPV strain and the CPV field strain is possible if the two viruses will infect the same cell, which is unlikely. This event is just as natural as a recombination between two field strains and it is not to be expected that a recombinant will have virulence factors, which are not already present in the contributing parent field strain. CDV is not readily amenable to recombination.

# Part 4 – Efficacy

## *Introduction and general requirements*

According to the approved claim, Nobivac DP Plus is intended for the active immunisation of puppies from four weeks of age onwards

- to prevent clinical signs and mortality of canine distemper virus infection and canine parvovirus infection and
- to prevent viral excretion following canine distemper virus infection and following canine parvovirus infection.

Onset of immunity: for canine distemper virus: 7 days
for canine parvovirus: 3 days
Duration of immunity: 8 weeks

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC as amended, and the Ph. Eur. monograph 0062 (Vaccines for veterinary use), Ph. Eur. chapter 5.2.7 as well as Ph. Eur. monographs 0448 (Canine Distemper vaccine - live) and 0964 (Canine Parvovirosis vaccine - live).

## *Challenge model:*

The efficacy of CDV and CPV components of Nobivac DP Plus was assessed by challenges with heterologous challenge strains according to component-specific monographs.

CDV challenge strain

CDV strain Snyder Hill was isolated in 1950 in the United States from a dog showing generalised signs of distemper (Gillespie and Rickard 1956). Virus derived from the original isolate was used by Chalmers and Baxendale (1994) to challenge dogs vaccinated with Nobivac vaccine containing CDV strain Onderstepoort from the same lineage. The challenge material used in the studies provided for Nobivac DP Plus derived from the challenge virus used by Chalmers and Baxendale.

To validate the challenge model, one study was performed.

Five puppies, seronegative against CDV, were divided into two groups and challenged at 5 weeks of age:

Group 1: 2 puppies, challenged Snyder Hill virus [1 ml intravenously (i.v.)]

Group 2: 3 puppies, challenged Snyder Hill virus (1 ml i.v.)

After challenge, clinical signs, rectal temperature and body weights were recorded daily. Pathognomonic clinical signs of disease (malaise, reduced appetite, gastrointestinal and respiratory signs, tremor, weight loss) were seen in the pups of both groups. Virus was isolated post-challenge from nasal and conjunctival swabs of all puppies of group 1 and of 2/3 of group 2. The amount of virus used in group 1 was chosen since it induced pathognomonic signs of disease in all control animals. The challenge model is considered valid.

In the same study, the efficacy of the CDV Onderstepoort vaccine strain was assessed.

Five puppies were divided into two groups:

Group 3: 2 puppies were vaccinated with Nobivac DHPPi + Nobivac L4 at 4 weeks and 6 days of age (CDV titre: $10^{5.0}$ $TCID_{50}$/ml). Seven days later they were challenged with Snyder Hill challenge strain.

Group 4: 2 controls, only vaccinated with Nobivac L4.

After challenge, clinical signs, rectal temperature, body weight, nasal and conjunctival swabs were taken daily. After challenge, both control pups had transient pyrexia, weight loss and clinical signs of disease (malaise, reduced appetite). One puppy had gastrointestinal distress and was euthanised. As the disease progression was somewhat delayed in comparison to previous studies, neurological disturbance was not seen in the controls. Due to facility constraints, the study design was not optimal, and the challenge phase was terminated 9 days after challenge. All pups were seronegative for CDV before vaccination and challenge. The vaccinates seroconverted by 16 days after vaccination and 9 days after challenge. Virus was isolated from the nasal and conjunctival swabs of one control puppy. No virus could be isolated from swabs taken from vaccinated pups.

It can be concluded that the canine distemper Onderstepoort vaccine strain provides a 7-day onset of immunity when administered from 4 weeks of age. As the challenge phase was not long enough this part of the study can be considered as information only.

CPV challenge strain

For CPV challenges, a pathogenic Italian 2c strain (136/006) was used. The pathogenicity of this strain was described by Spibey et. al. (2008). For all challenges the same dose of challenge virus was given by the oral route as described by Spibey et. al. This CPV challenge model was in use by the company for many years. Therefore, no further validation studies were performed. This is considered acceptable.

## *Efficacy parameters and tests:*

The 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 2001/82/EC as amended), in Ph. Eur. monographs 5.2.7. "Evaluation of efficacy of veterinary vaccines", 0062 "Vaccines for veterinary use", 0448 "Canine Distemper vaccine (live)" and 0964 "Canine Parvovirosis vaccine (live)" were followed.

The challenge studies contained vaccine groups and for every vaccine group a corresponding unvaccinated control group. The results of the control and vaccine groups were evaluated according to the requirements of the specific Ph. Eur. monographs.

In addition, blood sampling was performed on several occasions during the studies. The serum was tested for antibodies against CDV (virus neutralisation test, VN test) and CPV (hemagglutination inhibition test, HAI and serum neutralisation test, SN test). The results of the vaccine group and control group were compared.

The test parameters can be regarded as adequate. Methods and appropriate validations for serological tests used in the clinical studies (the VN assay for CDV and the HAI test for CPV) are provided and are considered acceptable.

## *Efficacy documentation*

Seven studies were conducted to investigate the efficacy of the product and included 6 laboratory studies and one field trial. Laboratory studies were well documented and carried out in puppies of the minimum age recommended for vaccination, using batches containing the minimum proposed titre of the CDV and CPV antigens. Production batches were used in the field trial.

Overview of the submitted efficacy studies:

| Study title |
|---|
| Study to investigate the efficacy of an improved puppy DP vaccine in 4-week-old pups: canine distemper onset of immunity |
| Study to investigate the efficacy of an improved puppy DP vaccine in 4-week-old pups: canine parvovirus 3 days onset of immunity |
| Study to investigate the efficacy of an improved puppy DP vaccine in 4-week-old pups: canine distemper duration of immunity |
| Study to investigate the efficacy of an improved puppy DP vaccine in 4-week-old pups: canine parvovirus duration of immunity |
| Efficacy of the CDV Onderstepoort strain in 4-week-old pups with maternally derived antibodies |
| Efficacy of the CPV 630a strain in 4-week-old pups with maternally derived antibodies |
| A clinical study in Morocco to assess the safety and efficacy of a new Nobivac CPV2c/CDV vaccine in puppies |

## *Laboratory trials*

## *Dose determination*

As regards the CPV component of Nobivac DP Plus one study was performed to determine the minimum immunisation dose.

In this study, the minimum effective dose for the CPV component of Nobivac DP Plus was established.

Twenty-one (21) SPF dogs, 10 weeks old, were divided into seven groups and vaccinated subcutaneously with 1 ml of the test article CPV 630a:

Group 1: 3 dogs, $10^5$ $TCID_{50}$ of test article CPV 630a
Group 2: 3 dogs, $10^{5.5}$ $TCID_{50}$ of test article CPV 630a
Group 3: 3 dogs, $10^6$ $TCID_{50}$ of test article CPV 630a
Group 4: 3 dogs, $10^5$ $TCID_{50}$ of test article CPV 630a
Group 5: 3 dogs, $10^{5.5}$ $TCID_{50}$ of test article CPV 630a
Group 6: 3 dogs, $10^6$ $TCID_{50}$ of test article CPV 630a
Group 7: 3 dogs, unvaccinated controls

All dogs were challenged orally with CPV-2c Italian field virus:

Groups 1, 2, 3 three days after vaccination; Groups 4, 5, 6 five days after vaccination.

Blood samples for serology were taken on D0, 5, 7, 11/12, 28; in groups 1, 2, 3 and 7 additionally on D2. Blood samples for white blood cell count were taken on D-1, -3, the day of challenge and D8, 10, 12 and 15 post-challenge. Clinical observation was performed daily, and the puppies were weight every day.

Rectal swabs were taken from D-1 until D21.

After challenge, there were no clinical signs of parvovirus infection in any of the vaccinates. With the exception of one dog, all rectal temperatures remained within normal parameters and did not exceed 39.5 °C. One dog had a temperature of 39.5 °C on D20; however, it is unlikely that this represents a challenge-related pyrexia. All dogs gained weight throughout the study. All vaccinates shed vaccine virus over a period of one to four days. The level or duration of shedding does not appear to correlate with the vaccine dose. The pups in groups 1, 2 and 3 begin to shed vaccine virus two days post vaccination, the pups in groups 4, 5 and 6 begin to shed vaccine virus four days post vaccination. The shedding of vaccine virus after challenge was confirmed by PCR. On the day of challenge, 2/3 dogs of groups 1 and 2, and all dogs of group 3 had begun to seroconvert in a dose-dependent manner. Two days post-challenge, all pups had strongly seroconverted.

All pups of groups 4, 5 and 6 seroconverted on the day of challenge. The antibody titres were maximal on D12 and remained high until the end of the study.

All control dogs were euthanised six days post-challenge with acute symptoms of haemorrhagic parvovirus infection. One dog had a spike in rectal temperature on D9 that coincided with the beginning of the symptoms. No pyrexia was seen in the other control dogs. All dogs gained weight until D9. In line with the beginning of clinical symptoms, they lost weight until the end of the study. All control pups began to shed field virus on D8; 3 days post challenge. The titres were maximal on D10 and remained high until the end of the study. Thymic hypoplasia was found in all control animals.

As regards the white blood cell count, 1/3 control animals showed a reduction > 50% of the baseline average but were euthanised before a full dataset could be obtained.

All pups were seronegative for CPV at the start of the study. They seroconverted 6 days post challenge.

In conclusion, the results of this study indicate that vaccination with CPV 630a with a titre of $10^5$ $TCID_{50}$ is able to provide a 3-day onset of immunity against clinical signs and challenge virus shedding in 10-week old SPF pups.

## *Onset of immunity*

Two studies were carried out in puppies in compliance with Ph. Eur. requirements to investigate the onset of immunity for the two antigens of Nobivac DP Plus.

**CDV**

Nine 4-week old SPF puppies, seronegative against CDV, were divided into two groups:

Six puppies were vaccinated on D0 with Nobivac DP Plus (subcutaneously, CDV potency $10^{5.1}$ $TCID_{50}$/dose) and Nobivac KC (intranasally), three control puppies were vaccinated on D0 with Nobivac KC (intranasally) only. Seven days after vaccination all dogs were challenged intravenously with canine distemper challenge virus Snyder Hill. The puppies were observed daily from D-1 to D28. Rectal temperature and body weight were determined daily from D-1 to D28. Blood samples for serology were taken on D-1, 7, 28 for all dogs and on D10 and 17 for the vaccinates. Nasal and conjunctival swabs were taken on D0, 7, 10, 13, 15, 17, 20, 22, 24 and 28.

From vaccination to challenge all vaccinates and controls did not show any clinical signs, had temperatures in the physiological range (37.9 °C – 39.4 °C) and gained weight. As regards CDV, they were seronegative on the day of challenge. As regards CPV, the vaccinates had seroconverted on D7. The titres remained high until the end of the study. The controls remained seronegative throughout the study.

After challenge, one vaccinated puppy showed coughing and clear serous nasal discharge on study D13 and clear serous nasal discharge on D14. Another puppy coughed on D4 and vomited on D16. A third dog vomited on D19. All other vaccinates showed no clinical signs until the end of the study. All vaccinated puppies had temperatures in the physiological range and gained weight until the end of the study. The nasal and conjunctival swabs of the vaccinates were negative for CDV. Three days after challenge, the puppies seroconverted regarding CDV. The titres increased until the end of the study.

All control puppies showed severe clinical signs of distemper (coughing, sneezing, nasal discharge, malaise, reduced appetite, vomiting) starting 6 days after challenge. One puppy had respiratory clinical signs until the end of the study. The other two puppies developed neurological signs (tremor, chorea, twitching). One puppy had to be euthanised on study Day 16. A slight increase in temperature was noted in all three puppies after challenge. One puppy had pyrexia on study D16. Two puppies lost weight over two or more consecutive days. Viral shedding began 6 to 8 days (nasal) or 8 days (conjunctival) after challenge in 2/3 or 1/3 animals, respectively.

The two controls that survived the challenge had CDV titres at the end of the study.

The study was designed to meet the requirements of Ph. Eur. monograph 0448. However, two deviations were made:

- As the use of Nobivac DP Plus is intended for puppies of 4 weeks of age onwards, the age of the animals used in this study was 4 weeks of age instead of 8-16 weeks.
- As a short onset of immunity is applied for this vaccine, the time between vaccination and challenge was 7 days instead of 20-22 days.

These deviations from the immunogenicity test stated in the monograph are considered acceptable as they can be regarded as a worst-case scenario.

According to Ph. Eur. monograph 0448, the challenge is not valid if during the observation period after challenge fewer than 100% of the control dogs die or show notable signs of canine distemper. In this study, all control dogs developed severe clinical signs of distemper and one puppy had to be euthanised. Therefore, this requirement of the monograph is fulfilled, and the challenge is considered valid.

Ph. Eur. monograph 0448 states that ”the vaccine virus complies with the test if during the observation period after challenge all the vaccinated dogs survive and show no signs of disease.”

In this study, one puppy showed an incidental cough and clear serous nasal discharge on study D13 and D14. Another puppy coughed on D14 and vomited on D16. A third dog vomited on D19.

The applicant justified that these clinical signs are not related to the challenge as it is quite normal for puppies in this age to show these symptoms as they are continuously exposed to micro-organisms (food, mother, animal handlers, toys). Moreover, dogs are enthusiastic eaters and often eat too fast or too much, leading to the occasional vomit. Furthermore, the puppies in this study were concurrently vaccinated with Nobivac KC. Above observed clinical signs are described in section 4.6 of the SPC of Nobivac KC. Therefore, it is not finally possible to decide if the observed clinical signs of the vaccinates (coughing and nasal discharge) are attributable to the vaccination with Nobivac KC or to the challenge virus. In addition, as CDV challenge virus could not be isolated from the conjunctiva or upper respiratory tract of the vaccinates it is unlikely that the clinical observations are related to an infection with CDV. Therefore, the proposed claims “to prevent clinical signs” “prevention of viral shedding” and “to prevent mortality” are supported by this study.

**CPV**

Twelve 4-week old SPF puppies, seronegative against CPV, were divided into two groups:

Seven puppies were vaccinated on D0 with Nobivac DP Plus (subcutaneously, CPV potency $10^{5.1}$ $TCID_{50}$/ dose), five control puppies did not receive a vaccination. Three days after vaccination all dogs were challenged orally with Italian CPV type 2c field strain. The puppies were observed daily from D-1 to D17. Rectal temperature and body weight were determined daily from D-1 to D17. Blood samples for serology and white blood cell count were taken on D-1, 0, 1, 3, 6, 8, 10, 13, 17. Rectal swabs were taken daily from D-1 to D17.

From vaccination to challenge all vaccinates and controls did not show any clinical signs, had temperatures in the physiological range (37.9 °C – 39.4 °C) and gained weight. Vaccine virus (confirmed by PCR) was isolated from the rectal swabs of the vaccinates over a one to five-day period from 2 to 6 days post vaccination. The swabs of the control dogs were negative for CPV until challenge.

All puppies were seronegative against CPV and CDV prior to vaccination. As regards CDV the vaccinated puppies showed a clear serological response at the end of the study. As regards CPV on the day of challenge (3 days after vaccination) all vaccinates showed a low seroconversion in the HAI and SN test.

After challenge, all vaccinated puppies did not show any clinical signs of CPV disease until the end of the study. They had temperatures in the physiological range and gained weight until the end of the study. No challenge virus was shed by any of the vaccinates. There was no evident leukopenia during the study. The serological response to CPV reached maximum levels by study D6 and D10 and remained high until the end of the study.

All control puppies showed severe clinical signs of canine parvovirus disease (quiet behaviour, malaise, lack of appetite, mucoid/haemorrhagic diarrhoea, dehydration, vomiting) starting 4 days after challenge. The puppies were euthanised 5 or 6 days after challenge. A transient pyrexia was observed in 3/5 puppies. All puppies lost weight over multiple consecutive days. Virus was isolated from the rectal swabs over a three to four-day period from 3 to 6 days post challenge. All controls shed high levels of field virus on D9, the day of euthanasia. Leukopenia was seen in 1/5 puppies and 3/5 puppies showed a low white blood cell count below the level considered normal for a dog. Since all control dogs were euthanised 5 or 6 days after challenge, it was not possible to obtain data until the end of the study. Seven days after challenge, all controls had seroconverted.

The study was designed to meet the requirements of Ph. Eur. monograph 0964. However, one deviation was made: As a short onset of immunity is applied for this vaccine, the time between vaccination and challenge was 3 days instead of 20-22 days. This deviation from the immunogenicity test stated in the monograph is considered acceptable as it can be regarded as a worst-case scenario.

This study fulfils the requirements of the monograph. Therefore, the proposed onset of immunity of 3 days is considered acceptable.

## *Duration of immunity*

Two studies were carried out in puppies to investigate the duration of immunity for the two antigens of Nobivac DP Plus.

**CDV**

Eleven 4-week old SPF puppies, seronegative against CDV, were divided into two groups:

Group 1 (6 puppies) was vaccinated on D0 with Nobivac DP Plus reconstituted in Nobivac L4 (subcutaneously, CDV potency $10^{5.1}$ $TCID_{50}$/ dose) and Nobivac KC (intranasally). Group 2 (5 control puppies) was vaccinated on D0 with Nobivac L4 (subcutaneously) and Nobivac KC (intranasally). Four

weeks after the first vaccination all dogs of groups 1 and 2 received a second vaccination with 0.5 ml Nobivac L4. 8 weeks after the first vaccination all dogs were challenged intravenously with canine distemper challenge virus Snyder Hill (/dog). The puppies were observed daily from D54 to D77. Rectal temperature and body weight were determined daily from D54 to D77. Blood samples for serology were taken on D0, 28, 56 and 77. Nasal and conjunctival swabs were taken on D0, 56, 59, 62, 64, 66, 69, 71, 73, 77.

One vaccinated puppy showed lachrymation on several days after challenge. Another puppy had a petechial chest rash on several days after challenge. A third dog showed lachrymation and a petechial chest rash on several days after challenge. All other vaccinates remained healthy until the end of the study. All vaccinated puppies had temperatures in the physiological range and gained weight until the end of the study. The nasal and conjunctival swabs of the vaccinates were negative for CDV. All vaccinates had seroconverted on D28 with high titres. The titres remained high until the end of the study. As regards CPV, all vaccinates had seroconverted on D28 with high titres. The titres remained high until the end of the study. All vaccinates remained seronegative against CPiV throughout the study. All vaccinates had seroconverted against *B. bronchiseptica* on the day of challenge. As regards *Leptospira* (*L.*) Australis, Grippotyphosa and Canicola all animals of group 1 had seroconverted on the day of challenge. Regarding *L.* Icterohaemorrhagiae the dogs of group 1 did not show any seroconversion.

All control puppies showed severe clinical signs of distemper: respiratory/gastrointestinal phase with diarrhoea, coughing, malaise, reduced appetite, vomiting starting 4 days after challenge and epithelial phase with petechial abdominal and/or groin rash starting 10 days after challenge. Transient pyrexia was noted in all puppies several days after challenge. Sustained weight loss over multiple consecutive days was seen in each puppy during the first phase of the disease. Viral shedding began 3 to 6 days (nasal) or 3 to 8 days (conjunctival) after challenge and was detectable over a 3 to 8 days period. As regards CDV the animals remained seronegative until the challenge and had seroconverted at the end of the study. Regarding CPV the control animals remained seronegative until the end of the study. The control animals were seropositive against *B. bronchiseptica* on D0. The titres declined until challenge. It is likely that the dogs were infected with *B. bronchiseptica* during the transport, which took place two weeks before. The animals had seroconverted against CPiV on Day 28. The titres declined until challenge. It is likely that the infection with *B. bronchiseptica* caused tissue damage, which might be an aid in the opportunistic growth of the canine parainfluenza vaccine strain. This combination could have resulted in the enhanced antibody response. As regards *L.* Australis, Grippotyphosa and Canicola all animals of Group 2 had seroconverted on the day of challenge. Regarding *L.* Icterohaemorrhagiae 4/5 dogs of Group 2 had seroconverted on the day of challenge.

The study was designed to meet the requirements of Ph. Eur. monograph 0448.

According to this monograph the challenge is not valid if during the observation period after challenge fewer than 100% of the control dogs die or do not show notable signs of canine distemper. In this study, all control dogs developed severe clinical signs of distemper and one puppy had to be euthanised. Therefore, this requirement of the monograph is fulfilled, and the challenge is considered valid.

Ph. Eur. monograph 0448 states that "the vaccine virus complies with the test if during the observation period after challenge all the vaccinated dogs survive and show no signs of disease."

In this study, one puppy showed lachrymation on several days after challenge. Another puppy had a petechial chest rash on several days after challenge and a third puppy showed lachrymation and a petechial chest rash on several days after challenge. The applicant is of the opinion that these clinical signs are not related to the challenge. As lachrymation is observed in most of the puppies in all laboratory studies it can be concluded that it is not related to the CDV challenge. As regards the

abdominal rash observed in the vaccinates the applicant states that it can be regarded as a sign of an active immune system neutralising a low level of the challenge virus in the epithelium. The observation of a more severe rash in all of the surviving controls indicates a much higher level of virus replication and inflammation, in line with the clinical signs of systemic disease. This explanation is considered acceptable. Therefore, the requirement of the monograph is fulfilled and the proposed duration of immunity of 8 weeks is considered acceptable.

**CPV**

Ten 4-week old SPF puppies, seronegative against CPV, were divided into two groups:

Group 1 (7 puppies) was vaccinated on D0 with Nobivac DP Plus (subcutaneously, CPV potency $10^{5.0}$ $TCID_{50}$/dose) and Nobivac KC (intranasally). Group 2 (3 control puppies) was vaccinated on D0 with Nobivac KC (intranasally). 8 weeks after vaccination all dogs were challenged orally with Italian CPV type 2c field strain. The puppies were observed daily from D0 to D71. Rectal temperature and body weight were determined daily from D55 to D71. Blood samples for serology and white blood cell count were taken on D0, 6, 10, 17, 28, 49, 53, 55, 57, 60, 62, 64, 67, 71. Rectal swabs were taken on D0, then daily from D55 to D71.

All vaccinates did not show any clinical signs, had temperatures in the physiological range (37.9 °C – 39.4 °C) and gained weight. One vaccinated puppy showed lachrymation on D56, 62, 69, 70, 71. As lachrymation was also observed in the vaccination phase, it is not related to the challenge. The rectal swabs of all vaccinated puppies were negative for CPV. In group 1, there was no leukopenia evident during the study. All vaccinates had seroconverted against CDV on D17. The titres remained high until challenge. All vaccinates had seroconverted against CPV 2a, 2b and 2c on D6 with high titres. The titres remained high until the end of the study.

All control puppies showed typical clinical signs of canine parvovirosis. The first signs of disease began 3 to 4 days after challenge (malaise, soft faeces, vomiting). The symptoms rapidly progressed (loose mucoid/haemorrhagic diarrhoea, dehydration, abdominal pain, vomiting). Five days after challenge the dogs were euthanised on humane grounds. Transient increase in rectal temperature was noted in all puppies. Sustained weight loss over multiple consecutive days was seen in each puppy until they had to be euthanised. CPV was isolated over a period of three to five days, 1 to 5 days after challenge. All control dogs shed high levels of challenge virus until they were euthanised. Leukopenia (drop in white blood cell count below 50% of the pre-challenge baseline mean) was evident in none of the control pups. However, a drop in the white blood cell counts was seen five days after challenge. Since all of the control animals were euthanised on humane grounds on this day, it was not possible to obtain the full complement of white blood cell measurements. The control animals remained seronegative against CDV and CPV until challenge. After challenge, they developed antibody titres against CPV 2a, 2b and 2c.

This study fulfils the requirements of Ph. Eur. monograph 0964. The proposed claims "to prevent clinical signs and mortality of canine parvovirus infection and to prevent viral excretion following canine parvovirus infection" are supported by this study. Therefore, the proposed duration of immunity of 8 weeks is considered acceptable.

## *Maternally derived antibodies (MDA)*

Since Nobivac DP Plus is intended for puppies from 4 weeks of age onwards, two laboratory studies to investigate the influence of maternally derived antibodies on the efficacy of the vaccine were performed.

**CDV**

Sixteen 4-week old SPF puppies, seropositive against CDV, were divided into two groups:

Group 1 (10 puppies) was vaccinated on D0 with Nobivac DP Plus (subcutaneously, CDV potency $10^{5.1}$ $TCID_{50}$/dose). Group 2 (6 control puppies) was not vaccinated. The puppies were observed daily from D0 to D35. Blood samples for serology were taken weekly on D0, 7, 14, 21, 28 and 35. Nasal and conjunctival swabs were taken on D0. All puppies (vaccinates and controls) did not show any clinical signs during the study. The nasal and conjunctival swabs of all puppies (vaccinates and controls) were negative for CDV. Three puppies of group 1 seroconverted 14 days after vaccination. Their CDV titres on D0 were 16 and 40. Puppies with higher titres did not seroconvert after vaccination. Despite a history of frequent vaccinations, the mother of the puppies of group 2 had a low antibody titre on D0. Therefore, all her puppies had low maternally derived antibodies on D0. The puppies were seronegative on D7 and remained seronegative until the end of the study. The applicant is of the opinion that the low maternal antibody titres in the control pups do not invalidate the overall interpretation of the data from the vaccinates. As the live attenuated canine distemper Onderstepoort vaccine virus strain is not shed from the dogs after vaccination, the efficacy of vaccination in the presence of maternally derived antibodies can be assessed without the need for a control group.

The study indicated that the presence of >40 $VN_{50}$ units of maternally derived antibodies against CDV may influence the efficacy of the CDV component of Nobivac DP Plus. The relevance of this finding for the use of the vaccine in the field is, however, limited if no information is given about levels of MDAs expected in puppies from regularly vaccinated bitches, i.e. under normal field conditions. The study shows that vaccination with Nobivac DP Plus was able to break through in dogs with MDA levels $\leq$ 40 $VN_{50}$. However, only three of the 10 dogs that were included in the study had MDAs below $\leq$ 40 $VN_{50}$. Considering the low number of animals in the study the relevance of the cut-off level is uncertain. Therefore, more data on the levels of maternally derived antibodies against CDV present in young puppies (born from mothers which were vaccinated against CDV with different distemper vaccines) are provided. Data provided show that the majority of 4-6-week-old puppies have antibody titres ≤40 $VN_{50}$ units, but not all. It is advised in the SPC and package leaflet to vaccinate 6-week-old puppies with Nobivac DP Plus followed by vaccinations with other core vaccines against canine distemper, canine parvovirus, canine contagious hepatitis and respiratory disease caused by adenovirus type 2 infection. This information for the practitioner is considered acceptable.

**CPV**

Sixteen 4-week old SPF puppies, seropositive against CPV, were divided into two groups:

Group 1 (11 puppies) was vaccinated on D0 with Nobivac DP Plus (subcutaneously, CPV potency $10^{5.1}$ $TCID_{50}$/dose). Group 2 (5 control puppies) was not vaccinated. The puppies were observed daily from D0 to D59. Blood samples for serology were taken twice a week on D-8, 0, 4, 7, 11, 14, 18, 21, 25, 28, 31, 34, 38, 41, 45, 48, 52, 55, 59. Rectal swabs were taken on D0. All puppies (vaccinates and controls) did not show any clinical signs during the study. The rectal swabs of all puppies (vaccinates and controls) were negative for CPV. On the day of vaccination, the range of maternally derived antibodies against CPV-2c measured in the vaccinates was between 288 and 1664 HAI units and 806 and 4525 $SN_{50}$ units, respectively. All vaccinated puppies seroconverted prior to the decay of maternally derived antibodies in the controls. The first puppies seroconverted on D7. All puppies had seroconverted by D52. After seroconversion, the titres remained high until the end of the study. On the day of vaccination, the range of maternally derived antibodies against CPV-2c measured in the controls was between 288 and 832 HAI units and 640 and 4032 $SN_{50}$ units, respectively. The antibody titres declined continuously (half-life of approximately 11 to 14 days) until the end of the study.

In this study, it was demonstrated that the vaccine was able to break through HAI antibody titre levels against CPV-2c varying between 288 and 1664 HAI units and 806 and 4525 $SN_{50}$ units respectively. Therefore, it can be concluded that maternally derived antibodies against CPV do not have a negative influence on the immune response to vaccination with Nobivac DP Plus. This is reflected in section 5 of

the SPC: "Maternally derived antibodies against canine parvovirus do not interfere with the efficacy of this product."

## *Interactions*

As regards section 4.8 of the SPC the following wording is proposed:

*"Safety data are available which demonstrate that this vaccine can be administered on the same day but not mixed with vaccine of the Nobivac series containing Bordetella bronchiseptica and canine parainfluenza virus components for intranasal administration. Efficacy after concurrent use has not been tested. Therefore, while safety of concurrent use has been demonstrated, the veterinarian should take this into account when deciding to administer the products at the same time."*

This wording is considered acceptable.

## *Field trials*

One field study was performed in two private kennels in Morocco under GCP conditions. As no control group was included, it was an open-label study. 89 dogs of different breeds (Pinscher, Spitz, Bichon, Cocker Spaniel, Spaniel, Husky, German Shepherd crossed with Belgian Shepherd, German Wirehaired Pointer, German Short Hair, Dobermann, Rottweiler), 25-43 days of age, male and female were included in the study. In 2015 there were 3 cases of CPV infection in litters from one breeder and in 2016 there were cases of kennel-cough in litters from the other breeder. No new cases since then were noticed for both infections. As two puppies died during the study (1 Pinscher on Day 4 due to ascariasis, 1 Spitz on D12 due to haemorrhagic gastric ulcers) the results of 87 puppies were assessed. Two batches of Nobivac DP Plus were used (CPV potency: $10^{5.8}$ $TCID_{50}$/dose, CDV potency: $10^{5.2}$ $TCID_{50}$/dose and CPV potency: $10^{5.9}$ $TCID_{50}$/dose, CDV potency: $10^{6.4}$ $TCID_{50}$/dose). All dogs were vaccinated subcutaneously on D0 with Nobivac DP Plus mixed with Nobivac L4 (0.5 ml). Blood samples for serology and rectal swabs were taken on D-3 and D21. The mothers of the puppies were weighed on D-3 and D21 and blood samples for serology and rectal swabs were taken on the same days.

To assess the efficacy of Nobivac DP Plus the antibody titres against CDV and CPV were measured three weeks after vaccination. Seroconversion was determined based on an expected half-life of CDV and CPV MDA titres of approximately 10 days; i.e. the titre of the sample on D21 had to be higher than 25% (¼) of the titre of the sample taken on D-3 in order to count a puppy as having seroconverted. The choice for a half-life of CDV and CPV of 10 days was based on Wilson et al. (2014) and Pollock and Carmichael (1982), respectively.

For the HAI test performed in the applicant's laboratory < 8 (1 in 2 dilution) or < 16 (1 in 4 dilution) HAI units are the limits of detection.

For the VN test performed in the applicant's laboratory ≤ 4 $VN_{50}$ is the limit of detection. 79/87 (91%) puppies seroconverted to CPV and 86/87 (99%) puppies seroconverted to CDV after vaccination. The nine puppies that did not seroconvert were not those with the highest MDA levels. However, this is not unusual as under field conditions there are always some puppies that do not seroconvert after vaccination for other reasons than the interference of MDAs.

The field study was performed in Morocco, which is outside the EU. The applicant argues that the study was carried out by a contract research organisation in accordance with GCP as required by EU Directive 2001/82/EC. The vaccinated puppies were of breeds that are also kept in the EU and the housing and daily husbandry of the bitches with pups were also comparable to the EU. Therefore, the conditions were representative for the EU as required by EMEA/CVMP/852/99-FINAL.

The applicant chose 2 private kennels for the field study. However, only one litter from one kennel (3 Cocker Spaniel puppies) was included in the study. All other litters (84 puppies) came from the second kennel. The applicant explains that on the first site, the number of litters at the time of the study was less than expected at start of the study. This is considered acceptable.

According to EMEA/CVMP/852/99-FINAL, the trial shall, unless justified, compare a group of vaccinated animals with an equivalent group of unvaccinated or placebo controls. In this study, no control group was included. The applicant states that Nobivac DP Plus is a live vaccine and, as is the case for all live CPV vaccines for dogs in susceptible animals, the CPV component is shed and spread via the faecal-oral route. Preventing the spread of vaccine virus to e.g. unvaccinated control animals is virtually impossible in a field situation. As it was a combined safety and efficacy study, control animals could have become seropositive due to spread of the vaccine strain by vaccinated animals, which might have compromised the outcome with regard to the efficacy part of the study. Therefore, it was decided not to include a control group in this field study. This is considered acceptable.

In the CPV MDA laboratory study Nobivac DP Plus batch 12I17b was used. 4/11 puppies (36%) did not seroconvert within 21 days after vaccination but at a later time point (D28, 41 and 52). In the field study two different batches were used. Within 21 days after vaccination 47/47 puppies (100%) and 34/42 puppies (81%) seroconverted with batch CPC15.20.206Z and 25D17, respectively. However, the study was terminated on day 21. Therefore, it was not possible to detect an antibody titre increase at later time points.

## *Overall conclusion on efficacy*

Six laboratory studies and one field study were conducted to evaluate the efficacy of Nobivac DP Plus.

The minimum protective dose of $10^{5.1}$ $TCID_{50}$ for CDV and CPV could be demonstrated.

The onset of immunity of 7 days for CDV and of 3 days for CPV was shown as well as a duration of immunity of 8 weeks for both antigens.

As regards the efficacy of Nobivac DP Plus, the applicant proposes the following claims for the CDV component: "prevent clinical signs and mortality of canine distemper virus infection and prevent viral excretion following canine distemper virus infection". The claims are considered supported by the studies.

As regards the CPV component the proposed claims "to prevent clinical signs and mortality of canine parvovirus infection and to prevent viral excretion following canine parvovirus infection" are supported by the studies.

Since Nobivac DP Plus is intended for puppies from 4 weeks of age onwards, two laboratory studies to investigate the influence of maternally derived antibodies on the efficacy of the vaccine were performed.

Based on the results of these studies and additional data about the levels of maternally derived antibodies against CDV present in young puppies (born from mothers which were vaccinated against CDV with different distemper vaccines) the following is stated in section 4.4 of the SPC: "*Moderate to high levels of maternally derived antibodies against canine distemper virus can reduce the efficacy of the product against canine distemper".* In section 5 of the SPC it is stated that *"Immunity against canine distemper virus is achieved in animals of 4 weeks of age with low to moderate levels of maternal antibodies. Maternally derived antibodies against canine parvovirus do not interfere with the efficacy of this product."* This is considered acceptable.

It is proposed to allow the use of Nobivac DP Plus with the intranasal vaccine Nobivac KC on the same day. However, the absence of sufficient data in support of efficacy for Nobivac KC when used this vaccine in combination with Nobivac DP Plus is stated in SPC section 4.8.

The advice for the mixed use with Nobivac L4 was deleted.

An advice for the practitioner on how to use this vaccine and when to continue the routine vaccination with other vaccines in order to achieve appropriate protection against the major infectious diseases of young dogs is included in the SPC and package leaflet.

# Part 5 – Benefit-risk assessment

## *Introduction*

Nobivac DP Plus is a bivalent live virus vaccine, which is indicated for the immunisation of healthy puppies from four weeks of age onwards by one subcutaneously injection to prevent clinical signs, mortality and viral excretion following canine distemper virus infection and to prevent clinical signs, mortality and viral excretion following canine parvovirus infection.

It is the successor vaccine of the nationally licensed product Nobivac Puppy DP and contains the same CDV strain Onderstepoort. The CPV strain, however, was replaced by the live CPV recombinant vaccine strain 630a.

Nobivac DP Plus is presented as a freeze-dried vaccine. Each vial contains one dose. Before use, the lyophilisate is reconstituted with 1 ml of solvent. No adjuvant or preservatives are included in this product.

The dossier was submitted in line with requirements of Article 12(3) of Directive 2001/82/EC.

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

The vaccine components CDV and CPV are directed against canine infectious diseases present and widespread in most European countries. The vaccine protects against distemper and parvovirosis which are serious illnesses and often fatal in young dogs.

Well-conducted controlled laboratory trials demonstrated that the product is efficacious. The following SPC claims are proposed by the applicant:

For the active immunisation of puppies from 4 weeks of age onwards to prevent clinical signs and mortality of canine distemper virus infection and canine parvovirus infection and to prevent viral excretion following canine distemper virus infection and following canine parvovirus infection.

Onset of immunity: for canine distemper virus: 7 days;
for canine parvovirus: 3 days.

Duration of immunity: 8 weeks.

A direct beneficial effect is seen based on the individual animal, which is protected from canine distemper and canine parvovirosis.

Two studies were performed to assess the possible influence of maternally derived antibodies on the antibody response to CDV and CPV. Regarding CDV a negative influence of maternally derived antibodies on the antibody response to CDV after vaccination was shown. Advice for the practitioner on how to use this vaccine and when to continue the routine vaccination with other vaccines in order to achieve appropriate protection against the major infectious diseases of young dogs is included in the SPC and package leaflet.

As regards the CPV component it could be shown that the maternally derived antibody titres did not have a negative influence on the active immunity against CPV. This property of the vaccine helps very young puppies to overcome the so-called "window of susceptibility" or "immunity gap" which is a dangerous period of time during which maternally derived antibodies have fallen below protective levels against virulent field strains but are still able to interfere with the efficacy of the vaccination. The very short onset of immunity (3 days for CPV and 7 days for CDV) is a benefit for those puppies, which do not have maternally derived antibodies and are therefore fully susceptible for infections with CDV and CPV.

## Additional benefits

As vaccination with Nobivac DP Plus leads to prevention of viral excretion following CDV infection and following CPV infection, the vaccination will not only prevent dog-to-dog transmission of field virus but also the transmission to other susceptible in-contact species.

## Risk assessment

Quality:

Development, manufacture and control of the active substance and finished product is well described and ensure a consistent quality of the finished product.

Safety:

The main potential risks are identified as follows:

*For the target species and non-target species:*

Subcutaneous injection of Nobivac DP Plus induces soft diffuse painless thickening at the injection sites up to 1 cm, which is normally resolved within 1 to 7 days post vaccination. During or immediately after vaccination whining and/or scratching at the injection site may occur.

After mixed administration with a leptospirosis vaccine, a transient reduction of white blood cells counts in circulating blood below 50% of their mean baseline value was observed on D3, 5 and 7 after vaccination. However, the mixed use of the vaccine with a leptospirosis vaccine is not further advised.

Studies have been carried out in pups of the most sensitive category of target animals, i.e. 4-week-old SPF pups.

Nobivac DP Plus is restricted to the infection of dogs and other animals of the family Canidae, as well as members of the family Felidae (e.g. domestic cats) and Procyonidea (e.g. raccoons). Accordingly, a warning has been included in section 4.5 of the SPC indicating that the vaccine virus may spread to cats after contact with primary vaccinated dogs or faeces of dogs and maybe also spread to other cats without the induction of clinical signs.

*Risk for the user:*

The risk of accidental self-injection of the vaccine to the user is very low. The consequences of accidental self-injection are negligible. In addition to it, there are no user safety issues identified.

*Risk for the environment:*

As regards the assessment in accordance with Directive 2001/18/EC, all points to be considered for a live recombinant vaccine have been addressed. In respect of the live CPV recombinant vaccine strain 630a, the GMO poses no enhanced risk for target and non-target animals, environment and humans even though the vaccine virus is transferred via the faeces of a vaccinated dog to another animal.

There are no indications that recombination or reversion to virulence occurs under field conditions. Therefore, the risk that a hazard occurs is considered as low.

For the CDV component there are no potential risks for the environment since this strain is not shed by vaccinated animals. The CDV strain is derived from the same seed lineage as the CDV that has been used for a long period by the company as a live vaccine strain. Environmental problems and reversion to virulence have not been reported for CDV derived from this lineage. Therefore, the risk that a hazard occurs is considered as negligible.

## *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 and environment and to provide advice on how to prevent or reduce these risks. A warning for the contact of cats with recently vaccinated pups or their faeces and spreading to other cats in alignment with the results of the non-target safety studies is included in the SPC under section 4.5.

Environmental safety:

The assessment of level of risk for the environment for Nobivac DP Plus can be considered as low. Second phase evaluation is not considered necessary. The environmental risk assessment in accordance with the principles of Annex II of Directive 2001/18/EC is considered low.

## *Evaluation of the benefit-risk balance*

Based on the available date, the CVMP concludes that the benefit-risk balance is positive.

## *Conclusion*

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Nobivac DP Plus is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.