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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Mhyosphere PCV ID (EMEA/V/C/005272/0000)

Vaccine common name: Mycoplasma hyopneumoniae and porcine circovirus vaccine

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

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Introduction

The applicant Laboratorios Hipra, S.A. submitted on 23 April 2019 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Mhyosphere PCV ID, through the centralised procedure under Article 3(1) of Regulation (EC) No 726/2004 (mandatory scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 24 January 2019 as Mhyosphere PCV ID has been developed by recombinant DNA technology.

The approved indications are:

“For the active immunisation of pigs:

- to reduce lung lesions associated with porcine enzootic pneumonia caused by *Mycoplasma hyopneumoniae* and to reduce the incidence of these lesions (as observed in field studies).
- to reduce viraemia, virus load in lungs and lymphoid tissues and the duration of the viraemic period associated with diseases caused by Porcine circovirus type 2 (PCV2). Efficacy against PCV2 genotypes a, b and d has been demonstrated in field studies.
- to reduce culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases (as observed at 6 months of age in field studies).

M. hyopneumoniae: Onset of immunity: 3 weeks after vaccination. Duration of immunity: 23 weeks after vaccination.

Porcine circovirus type 2: Onset of immunity: 2 weeks after vaccination. Duration of immunity: 22 weeks after vaccination.

In addition, a reduction in nasal and faecal shedding and the duration of nasal excretion of PCV2 was demonstrated in animals challenged at 4 weeks and at 22 weeks after vaccination.”

Mhyosphere PCV ID consists of the inactivated recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon, expressing the PCV2 capsid protein (CP). Thus, the vaccine is intended to protect against two swine pathogens at the same time. For *M. hyopneumoniae* and PCV2 CP the relative potency (RP) is ≥1.3 determined by ELISA tests. The vaccine is adjuvanted with light mineral oil. No preservative is added. The target species is pigs. The product is intended for administration by intradermal use as a single dose of 0.2 ml to pigs from 3 weeks of age.

Mhyosphere PCV ID is an emulsion for injection and is presented in 20 ml polyethylene terephthalate (PET) vials (containing 10 ml) with 50 doses and 50 ml PET vials with 100 doses (20 ml), 125 doses (25 ml) or 250 doses (50 ml).

The CVMP considers that recombinant *Mycoplasma hyopneumoniae*, strain 7304 (Nexhyon), expressing the capsid protein of porcine circovirus type 2a, is a new active substance.

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

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

The applicant received scientific advice (SA) from the CVMP on September 2015. The scientific advice pertained to the quality and clinical development sections of the dossier. The applicant deviated from the SA in two aspects: the challenge method used in efficacy studies and the vaccine formulation at a fixed volume of antigenic fraction. Adequate justifications were provided for the deviations from the SA.

MUMS/limited market status

Not applicable.

Multi-strain dossier

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

A detailed description of the pharmacovigilance system (dated 14/01/2019) 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

Manufacture of the final product takes place in the European Union at two sites of Laboratorios Hipra, S.A., both located in Amer, Girona, Spain. The manufacturing of the active substance takes place at Hipra, S.A. site Carretera C-63. Quality control of the active substance and manufacture of the final product including secondary packaging and batch release is carried out at the Laboratorios Hipra, S.A. site Avda. la Selva. Both sites have a manufacturing authorisation issued on 21 October 2017 by the Spanish competent authority (Agencia Española de Medicamentos y Productos Sanitarios, AEMPS).

GMP certificates which confirm the date of the last inspection (21 September 2018), are valid and show that both sites, Laboratorios Hipra, S.A. site Avda. La Selva (certificate issued on 08 May 2019) and Hipra, S.A. site Carretera C-63 (certificate issued on 30 April 2019), are authorised for the manufacture and batch release of such veterinary dosage forms, have been provided.

A GMP declaration for the manufacturing site of the active substance was provided from the Qualified Person (QP) at the EU batch release site. The declaration was based on an on-site audit of the active substance manufacturer.

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 substance and of the finished product manufacturing sites has been satisfactorily established and is in line with legal requirements. A list of organisms handled at the manufacturing sites has been provided.

Part 2 – Quality

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

The finished product is presented as emulsion for injection containing inactivated recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon. The vaccine contains *M. hyopneumoniae* with an RP (relative potency) ≥1.3 and PCV2 CP with an RP ≥1.3 per dose of 0.2 ml. The product is adjuvanted with light mineral oil.

Other ingredients are poloxamer 407, polysorbate 80, sorbitan monooleate, sodium hydroxide, manganese sulfate monohydrate, disodium edetate (EDTA), disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride and potassium chloride are included as excipients, as well as water for injections. The vaccine does not contain a preservative.

The product is available in 20 ml PET vials (containing 10 ml) with 50 doses and 50 ml PET vials with 100 doses (20 ml), 125 doses (25 ml) or 250 doses (50 ml).

Container and closure

The PET containers comply with European Pharmacopoeia (Ph. Eur.) chapter 3.2.2 and Ph. Eur. chapter 3.1.15. The vials are closed with chlorobutyl rubber stoppers (type 1) and aluminium seals. The rubber stoppers comply with Ph. Eur. chapter 3.2.9. The sterilisation processes applied are suitable to ensure sufficient innocuousness with respect to the risk of contamination due to container materials.

The pack /container sizes are consistent with the vaccination schedule and intended use.

Product development

The applicant has provided adequate information on the choice of antigenic fraction, adjuvant, excipients, container-closure system and overages as well as the vaccine production. Reasonable justification is given regarding the suitability of the chosen recombinant vaccine strain Nexhyon and the development of *in vitro* methods to test the potency of batches.

M. hyopneumoniae and PCV2 are among the most economically important pathogens for the swine industry and are frequently isolated as co-infectors in growing and finishing pigs. Although most *M. hyopneumoniae* infections are not severe, animals infected by *M. hyopneumoniae* are predisposed to secondary infections of the respiratory system that can reduce the daily weight gain rate or even cause death. PCV2 is a very small, non-enveloped, single-stranded circular DNA virus that belongs to the genus Circovirus. PCV2 is the causative agent of several diseases and syndromes, collectively referred to as porcine circovirus diseases (PCVD). Among them, the systemic disease caused by

PCV2 (PCV2-SD), formerly known as the post-weaning multisystemic wasting syndrome (PMWS), is the most significant. It is characterised mainly by loss of weight, general wasting, and severe immunosuppressive effects with premature death of piglets.

A wild type strain, isolated from the lung of a fattening pig with typical enzootic pneumonia lung lesions, was chosen as parental strain to obtain the recombinant vaccine strain which is able to express the PCV2 CP thereof. The parental strain was genetically modified using a plasmid, which included suitable promoter regions for further expressing exogenous DNA sequences of interest, in this case the capsid protein of PCV2. This recombinant *M. hyopneumoniae* strain, named Nexhyon, is able to express a nucleotide sequence coding for the PCV2 CP . The choice of the PCV2 synthetic capsid gene in the context of the porcine circovirus 2 genetic variability, epidemiology and biologic features and the choice of *M. hyopneumoniae* vaccine strain in the context of antigenic variability/virulence have been sufficiently justified.

For intradermal administration a powerful adjuvant is needed because its quantity is limited by the small volume per dose of only 0.2 ml. Mineral oil was chosen as it is known to induce a long lasting strong humoral and cellular immune response.

PET containers are useful when dealing with multi-dose containers because they are unbreakable and easier to handle by the final user. In addition, the PET containers chosen are clear and colourless which permits the visual inspection of the content.

The manufacturing process of the antigenic fraction was designed following the seed lot system, in accordance with the general Ph. Eur. monograph requirements on vaccines for veterinary use (Ph. Eur. 0062). Inactivation is carried out by a physical process which results in both the inactivation of the bacterium and the release of the PCV2 CP from the *M. hyopneumoniae* cytoplasm at the same time. The *M. hyopneumoniae*^{cpPCV2} culture is centrifuged twice to obtain a concentrate, which is reconstituted with sterile water for injections. The inactivation by a physical process is carried out with constant agitation for several days. The inactivation period was shortened during the registration procedure, the applicant provided new data. An inactivation kinetics study was conducted to demonstrate that the time required for inactivation does not exceed 67% of the total duration of the inactivation process. An inactivation control is carried out on each batch just after the inactivation process.

The *in vitro* methods used to test the potency of the vaccine consist of two capture ELISAs (Enzyme-Linked Immunosorbent Assays), one for *M. hyopneumoniae* and one for PCV2 CP. Both ELISA techniques are able to quantify immunorelevant regions of either *M. hyopneumoniae* or PCV2 CP by using suitable monoclonal antibodies. The antigen quantification is based on the calculation of the relative potency of the tested batch in comparison to a reference vaccine, which has demonstrated to be efficacious by means of challenge in the target species.

Description of the manufacturing method

The manufacturing process of the antigenic fraction was designed following the seed lot system, in accordance with the general Ph. Eur. monograph requirements on vaccines for veterinary use (Ph. Eur. 0062). The manufacturing process consists of the following main steps: Working seed bacteria are propagated at +36 to +38 °C in liquid culture medium (scaling up) and then transferred into a fermenter. Once a harvest is obtained, part of this harvest can be left in the same fermenter and the process of propagation starts again by adding fresh medium. This process can be repeated twice starting from the previous harvest. Afterwards, the harvest is concentrated by continuous centrifugation to eliminate the culture medium. The sediment is collected in a sterile recipient and brought up to volume with water for injections. Subsequently, the concentrated antigenic fraction is

filled in single use plastic bags where it is inactivated under orbital agitation at +2 to +8 °C for several days by means of a physical process. The inactivated antigen suspension can be stored at +2 to +8 °C for a maximum of 12 months until being further processed.

The final vaccine is prepared by blending the different components of the vaccine. Subsequently, the blend is emulsified by means of a high-pressure homogeniser and filled into PET vials under aseptic conditions and under continuous stirring.

Major steps of the manufacturing process have been validated by producing 3 consecutive batches. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in process controls established are adequate for this type of manufacturing process.

The production process is described adequately in the essential parts to ensure that the product will be of consistent quality and stable.

Production and control of starting materials

Starting materials listed in pharmacopoeias

Appropriate Certificates of Analysis are provided for each of the starting materials listed in pharmacopoeias. Internal specifications and/or representative certificates of analysis (CoA) have been provided and all conform to Ph. Eur. required specifications or their respective USP requirements.

Gelatin is a component used in the preparation of the seed stocks solution. The raw material used is exclusively of porcine origin. Due to its origin, no TSE risk can be deduced from this starting material. The risk of transmitting of extraneous agents is considered negligible bearing in mind the sterilisation treatments applied.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

Starting materials of biological origin, which are not listed in the Ph. Eur., are recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon, heart infusion (HI), PPLO broth, porcine serum, equine serum, and yeast extract.

For the active substance, a seed lot system was satisfactorily established. Details of source, passage history, controls, storage conditions for the master seed bacteria (MSB) and working seed bacteria (WSB) have been provided and are considered appropriate. Based on the risk assessment provided, the seed materials do not pose a risk for TSE transmission. Certificates of analysis for the MSB and WSB have been presented and further assurance has been provided that the seed materials are free from mycoplasma other than that of the vaccine strain. The construction of the vaccine strain has been sufficiently described. Satisfactory information regarding the source of the synthetic PCV2a capsid gene, the mechanism of incorporation of the genes into the genome of *M. hyopneumoniae* using a plasmid and the monoclonal antibody against PCV2a capsid protein were provided. Detailed

information on the antibiotic resistance gene and its risk present in the finished product were provided and considered acceptable.

Heart infusion (HI) and PPLO broth complies with the current regulatory texts related to Ph. Eur. monograph 5.2.8 “Minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products” and the TSE Note for Guidance EMEA/410/01-Rev.3. Valid TSE certificates of suitability have been provided for the stated suppliers. For the elimination of extraneous agents, the HI and PPLO broths undergo a High Temperature Short Time (HTST) treatment before adding them to the culture media. A validation was performed using model viruses covering a broad spectrum of viruses with different characteristics and different resistance properties demonstrating that the treatment is able to achieve a virus reduction higher than 10^{-6} as required by Ph. Eur. monograph 5.1.1.

Porcine serum and equine serum do not pose a risk for TSE transmission due to their origin. Both sera are treated by γ-irradiation, which complies with Ph. Eur. 5.1.1 requirements. For all suppliers a certificate of irradiation, a GMP certificate of the irradiation plant and a dose mapping qualification report were provided. In addition, a model validation report regarding the effectiveness of virus inactivation by gamma irradiation has been provided. The viruses used for validation are considered acceptable as they represent different virus families having different degrees of resistance to gamma irradiation. The results of the validation study demonstrate that more than 6 log10 reduction is achieved. Based on this information, the risk of virus contamination and its transmission is considered negligible.

Compliance with Ph. Eur. 5.2.5 with respect to bacterial and fungal sterility can be concluded as all media containing serum are sterilised through a bacteria-retentive membrane prior to their use. Moreover, there are several scientific publications indicating that gamma irradiation of animal sera at 25 - 40 kGy significantly reduces the levels of many types of microorganisms including bacteria, fungi and mollicutes (*Mycoplasma sp*. and *Acholeplasma sp.).* Yeast extract is of non-animal origin and is sterilised before use either by heat treatment or by filtration during the preparation of the culture media.

Starting materials of non-biological origin

A certificate of analysis has been provided for phenolsulfonephtalein sodium salt and it conforms to in-house specifications.

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

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

Control tests during the manufacturing process

The applicant presented *in process* data for the manufacture of six industrial scale antigen bulks. During the manufacture of the antigen, the following tests are carried out: Gram stain, purity, relative luminescence units, viable count, test for complete inactivation, bacterial and fungal sterility and antigen quantification by means of HPLC-MS/MS for *M. hyopneumoniae* and by means of ELISA for PCV2 CP. Test descriptions and the limits of acceptance are presented. With respect to the

antigen quantification tests well-defined ranges have been established for both methods HPLC-MS/MS (*M. hyopneumoniae*) and ELISA (PCV2 CP). This is considered crucial for a consistent antigen amount per dose. The in process tests are deemed sufficient to control all critical steps in the manufacturing process. Validation studies have been provided for all key tests.

Control tests on the finished product

Finished product controls performed on the bulk vaccine are appearance, viscosity, pH value, concentration of mineral oil, identification and potency of *M. hyopneumoniae* as well as identification and potency of PCV2 CP. The filled product will be controlled for appearance, bacterial and fungal sterility and correct volume.

The descriptions of the methods used for the control of the finished product and the specifications have been provided and all release limits have been justified by valid data. The relevant test methods, i.e. potency, quantification of the adjuvant and sterility are satisfactorily validated. Upon request, further details regarding the validation of the HPLC used for determination of the mineral oil content and regarding the validation for viscosity testing have been provided. Both tests deem suitable for their purpose.

Regarding the control of the correct antigen amount, the applicant has developed two *in vitro* potency tests (capture ELISA techniques) which are suitable to detect immune-relevant structures for *M. hyopneumoniae* and PCV2 CP. Based on safety and efficacy considerations the specifications set for the potency tests have been adequately justified. The quantification of *M. hyopneumoniae* and PCV2 CP is based on the calculation of the relative potency of the tested batch in comparison to "low potency" reference vaccines, which have been demonstrated to be efficacious in the challenge trials.

The replacement procedures for critical reagents including the respective reference vaccines have been adequately described and are considered acceptable.

Overall, the proposed tests on the finished product are considered adequate to control the product quality.

Batch-to-batch consistency

The applicant presented final product data for the manufacture of 3 consecutive final product batches indicating a consistent composition of the finished product in a quantitative and qualitative manner. All these final batches have also been included in the stability studies. The process of blending of large-scale batches has been verified using the maximum blending volume as described in the dossier Part 2B.

Stability

Stability of active ingredient (bulk antigen)

Real time stability studies with three antigenic fraction batches were carried out. Based on the data provided it can be concluded that the antigenic fraction remains stable for 12 months when stored at +2 to +8 ºC in plastic bags.

Additionally, an experimental vaccine batch was formulated with an antigen batch manufactured 12 months before blending. For the time being, it can be concluded that the vaccine manufactured with aged antigen complies with the established specifications. The applicant commits to complete

stability data up to 27 months and to inform immediately the competent authorities in case of any out of specification result.

Stability of the finished product

The proposed shelf-life is indicated as 24 months when stored at +2 to +8 °C. Stability studies with three industrial scale batches of finished product manufactured according to Part 2B and filled in PET vials have been carried out.

For all currently available points in time, the tested batches complied with the specifications and there is no obvious decline in potency as demonstrated by statistical evaluation.

Overall, the results obtained up to date show that the vaccine remains within the established specifications throughout a storage period of 27 months, when the vaccine is stored at +2 °C to +8 °C. Therefore, the data provided so far are sufficient to justify a 24 months shelf life.

In use stability of the vaccine

As the vaccine does not contain any preservative, the vaccine is recommended for immediate use. There are different presentations (50 doses, 100 doses, 125 doses and 250 doses) which allow the user to adjust the vial size to the number of animals to be vaccinated in one vaccination course. The use of intradermal device will substantially limit the risk of contamination.

The omission of preservatives in vaccine compositions is highly appreciated and supported by the statement included in guideline III/3469/97 "Inclusion of antimicrobial preservatives in immunological veterinary medicinal products" referring to the desirability of excluding potentially toxic excipients from medicinal products where possible, and the emphasis on formulation, manufacturing methodology and GMP as a means of achieving an acceptable product.

A justification for the non-performance of in use stability studies is provided.

Overall conclusions on quality

Mhyosphere PCV ID consists of the inactivated recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon, expressing the PCV2 capsid protein (CP). Thus, the vaccine is intended to protect against two swine pathogens at the same time. The antigenic fraction is adjuvanted with light mineral oil. Poloxamer 407, polysorbate 80, sorbitan monooleate, sodium hydroxide, manganese sulfate monohydrate, disodium edetate (EDTA), disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, and potassium chloride are included as excipients, as well as water for injections. No preservative is added. The pharmaceutical form of the final vaccine is an emulsion for injection. One dose consists of 0.2 ml.

The applicant has provided a comprehensive description of the development of the product including the choice of antigenic fraction, adjuvant, excipients, container-closure system and overages as well as the vaccine production and of the validation of the production process. Reasonable justification has been provided regarding the suitability of the chosen recombinant vaccine strain Nexhyon and the development of *in vitro* methods to test the potency of batches.

The production process of the antigen is based on the seed lot system. The manufacturing process consists of the following main steps: propagation of the bacteria in liquid culture medium, centrifugation to eliminate the culture medium, filling in single use plastic bags and inactivation.. The inactivated antigenic fraction can be stored at +2 to +8 °C for a maximum of 12 months until being further processed. In general, the production process of the antigenic fraction is described adequately

in the essential parts. The level of detail is sufficient to conclude that the product will be of consistent quality and stable.

The starting materials are properly described. For the starting materials of animal origin further assurance has been provided, that they are either tested for or treated to ensure that there are no contaminants or that the treatment process ensures the removal of any potential risk caused by extraneous agents. Furthermore, compliance of starting materials of animal origin with TSE regulation has been shown. The risk that the final product may transmit TSE to the target animal was estimated as negligible. Sufficient details regarding the construction and control of the seed material *M. hyopneumoniae*^{cpPCV2} have been provided.

The in process tests are deemed sufficient to control all critical steps in the manufacturing process. Validation studies have been provided for all key tests. With respect to the antigen quantification test, well-defined ranges have been established for both methods HPLC-MS/MS (*M. hyopneumoniae*) and ELISA (PCV2 CP). This is considered crucial for a consistent antigen amount per dose.

The proposed tests on the finished product are considered adequate to control the product quality. Validation studies have been provided for all key tests, i.e. potency, quantification of the adjuvant and sterility. Regarding the control of the correct antigen amount, the applicant has developed two *in vitro* potency tests (capture ELISA techniques). The tests are suitable to detect immune-relevant structures for both, *M. hyopneumoniae* and PCV2 CP, respectively. Based on safety and efficacy considerations the specifications set for the potency tests have been adequately justified and replacement procedures for critical reagents including the reference vaccines have been well described. Test results of three consecutive production runs including *in process* data for the manufacture of six industrial scale antigen bulks, conforming to the *in process* and final product specifications are provided.

Results of three antigenic fraction batches demonstrate that the antigenic fraction remains stable for 12 months when stored at +2 to +8 °C in plastic bags. With respect to the final vaccine the data provided are sufficient to justify a 24 months shelf life when stored at +2 to +8 °C. As the product does not contain any preservative, the vaccine is recommended for immediate use. There are different presentations (50 doses, 100 doses, 125 doses and 250 doses) which allow the user to adjust the vial size to the number of animals to be vaccinated in one vaccination course. The use of intradermal device will substantially limit the risk of contamination.

Overall, it is considered that quality is fully acceptable.

Part 3 – Safety

Introduction and general requirements

Mhyosphere PCV ID consists of the inactivated recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon, expressing the PCV2 capsid protein (CP). Thus, the vaccine is intended to protect against two swine pathogens at the same time. The vaccine is presented as emulsion for injection in which the concentrated antigenic fraction (aqueous phase) is adjuvanted with the oily phase (light mineral oil and surfactants). No preservative is included. The vaccine is administered intradermally at the sides of the neck using a suitable needle-free device. One administration of a single dose is recommended for animals of 3 weeks of age onwards (single lifetime dose).

A full safety file in accordance with Article 12(3)(j) has been provided. Studies to determine the safety of the vaccine were performed in accordance with the Ph. Eur. monographs 0062 on vaccines

for veterinary use, Ph. Eur. chapter 5.2.6 on evaluation of safety of veterinary vaccines and immunosera, Ph. Eur. monographs 2448 on inactivated porcine enzootic pneumonia vaccine, Commission Directive 2009/9/EC amending Directive 2001/82/EC and VICH GL 44 on target animal safety for veterinary live and inactivated vaccines.

The application refers to scientific advice (EMA/CVMP/SAWP/412022/2015) with regard to the batch used in the laboratory safety study. In order to ensure maximum antigen content, it was agreed to further concentrate the antigenic fraction during production without changing the proportion of the other components of the vaccine in order to establish a worst-case scenario.

Safety documentation

Two safety studies were conducted to investigate the safety of the product and included one laboratory study investigating the safety of the administration of one dose and one multicentre field trial.

The vaccine was administered by the intradermal route, as recommended. The laboratory study was reported to be good laboratory practice (GLP)-compliant and carried out in target animals of the minimum age recommended for vaccination (piglets, 3 weeks of age) using a batch with concentrated antigenic fraction and consequently maximum antigen content (RP *M. hyopneumoniae* = 4.4, RP PCV2 = 18.8). Production batches were used in the field trial carried out according to good clinical practice (GCP) principles.

The following safety studies were carried out with Mhyosphere PCV ID:

	Study title	
	Safety of one dose of Mhyosphere PCV ID in piglets	
	Clinical evaluation of the efficacy and safety of Mhyosphere PCV ID	

Laboratory tests

Safety of the administration of one dose

The safety of administration of the vaccine Mhyosphere PCV ID was tested in a laboratory study according to the requirements of Ph. Eur. 0062, 5.2.6, 2448 and VICH GL 44 as well as in conformity with OECD principles on GLP. The applicant used a sufficient number of animals of the most sensitive category (12 three-week-old piglets), the recommended route of administration (intradermal) and the recommended application scheme (one dose) to investigate the safety of the product. An appropriate control group (12 three-week-old piglets) receiving a placebo (PBS) was also included. All study animals were free from antibodies against *M. hyopneumoniae* and PCV-2 before vaccination and the controls remained negative until the end of the study.

The study was randomised and blinded.

The use of a batch with concentrated antigenic fraction is seen as worst-case scenario. Therefore, this approach is supported for the evaluation of the safety profile of this product (as already examined in the scientific advice). However, the potency values are outside of the ranges validated for the respective potency tests. Therefore, the applicant provided assurance of the reliability of the potency test results for the batch used and concluded that the results are valid. Thus, there was no

impact on the conclusions of the studies.

The animals were observed and examined at least daily for signs of abnormal local or systemic reactions. Rectal temperature was measured the day before and at the time of administration, 4 hours later and during the following 4 days.

No animal showed abnormal local or systemic reactions or signs of disease or died from causes attributable to the vaccine. Therefore, the study is considered valid. No negative effect on the evolution of piglet weight was observed throughout this study. The necropsy was unremarkable in all study animals.

The post vaccination increase in rectal temperature compared to the baseline is deemed slight, with a maximum individual increase of 0.46 °C on day 3 post-vaccination. The temperatures returned to baseline 1 or 2 days later. Taking into account the results of the field trial, section 4.6 of the SPC states: 'This slight increase subsided spontaneously within 48 hours without treatment'.
The mean rectal temperatures in the groups were very similar. The maximum group difference was 0.21 °C on day 1. Towards the end of the study, a mean group increase was not observed, as all mean group temperatures were below the corresponding mean group baseline temperatures.

Mild local reactions were reported for all vaccinated animals post-vaccination with a two-episode sequence lasting for a maximum of 7 days without interruption. One animal showed a moderate inflammation (3–5 cm). All local reactions disappeared before day 21 of the study without treatment. All adverse reactions are adequately reflected in the product literature.

In conclusion, the vaccine Mhyosphere PCV ID is considered safe for administration of one dose, if used according to the instructions described in the SPC.

Safety of one administration of an overdose

No overdose studies are required for inactivated vaccines.

Safety of the repeated administration of one dose

No repeated dose studies are required for this vaccine, taking into account that the vaccination schedule consists of a single lifetime dose.

Examination of reproductive performance

No reproductive studies were provided as the product is not indicated to be used during pregnancy, lactation and/or by breeding boars. The following warning has therefore been included in section 4.7 of the SPC: 'The use is not recommended during pregnancy and lactation'.

Examination of immunological functions

No studies were conducted to investigate the effects of the product on immunological functions, but no adverse effects were observed in any of the safety or efficacy studies. It is therefore unlikely that this vaccine will have an adverse effect on immunological functions due to the nature of the product (inactivated recombinant vaccine).

Special requirements for live vaccines

Since Mhyosphere PCV ID is not a live vaccine, this section is not applicable.

User safety

The applicant has presented a user safety risk assessment, which has been conducted in accordance with CVMP guideline on 'User safety for immunological veterinary medicinal products' (EMEA/CVMP/IWP/54533/2006) and EMEA/CVMP/543/03-Rev.1.

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are accidental self-administration and dermal and/or oral exposure. The vaccine consists of the inactivated recombinant *M. hyopneumoniae*^{cpPCV2} strain Nexhyon expressing the PCV2 CP and therefore is not infectious to humans.

Most excipients including the adjuvant are commonly used in other vaccines and do not pose a risk for the user. The protein stabiliser manganese sulfate monohydrate (used also as an additive in animal food) is also not known to cause local or systemic harmful effects in human beings in the (low) concentration used in the vaccine.

Since the product contains mineral oil, the standard warning for mineral oil-containing vaccines is included in the product literature.

Based on the above risk assessment, the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

No study of residues has been performed. All substances included in the composition of the vaccine are listed in Annex 1 of Commission Regulation (EU) No 37/2010 or in the list of substances considered as not falling within the scope of Regulation (EC) No 470/2009. Consequently, a withdrawal period of zero days has been established.

However, the protein stabiliser, manganese sulfate monohydrate, is included in annex 1 to Regulation (EU) No 37/2010 for oral use only (Table 1, no MRL required) although the product in question is administered via the intradermal route. According to EMEA/MRL/334/97, manganese is an essential element and a normal component of the diet in humans, and the use in veterinary medicinal products will add negligible amounts to the manganese intake from its use as feed additive. The recommended dietary intake for pigs is 40 mg of manganese/kg feed. The applicant argued that the low amount of manganese sulphate applied once in a lifetime via the intradermal route has no pharmacological activity and is therefore out of the scope of Regulations (EU) No 37/2010 and (EU) No 470/2009. Considering that manganese is an essential element being part of the normal diet of pigs and the low dose which is applied via intradermal route per dose (compared to the recommended oral dose of 40 mg), this approach is considered acceptable.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

The applicant has not provided data investigating interactions of the vaccine with other veterinary

medicinal products and therefore proposes to include a statement in Section 4.8 of the SPC that 'No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be made on a case by case basis'.

Field studies

One pivotal multicentre randomised, double blinded, placebo-controlled study was conducted to evaluate safety and efficacy of Mhyosphere PCV ID.
The study was conducted on 7 commercial farms located in France (5) and Hungary (2) with historical records of clinical or subclinical PCV-2 and/or *M. hyopneumoniae*-related diseases with recently confirmed presence of at least one of the two pathogens and did adhere to GCP standards. Randomisation was performed by farm.

Pigs previously vaccinated against PCV2 and/or *M. hyopneumoniae,* animals suffering a severe infectious process during their lifetime, apparently healthy animals sharing a pen with animals with clinical signs compatible with an infectious disease at day 0 and animals in abnormal physical condition were excluded from the study.

The applicant used a sufficient number of animals and the recommended dose (0.2 ml), the route of administration (intradermal) and the application scheme to investigate the safety of the product. Standard vaccine batches were administered.

The study was well designed and conducted. During the whole study period (up to approximately 6 months of age), the overall safety (adverse events other than post-vaccination adverse events) was assessed in all animals (1253 vaccinates, 1254 controls). In a sub-group of 180 pigs (30 vaccinates and 30 controls of three farms), rectal temperature, local reactions and systemic reactions were recorded on the day before administration (rectal temperature only), at administration, 4 hours post administration and on the following two days or until clinical signs disappeared. The chosen parameters were appropriate to investigate the safety of the product.

None of the animals showed a systemic reaction after administration of the product and no adverse effect attributable to the administration of the product could be revealed throughout the field study.

Regarding the post vaccination rectal temperature progression, a slight increase in the mean rectal temperature of 0.28 °C was observed in the vaccinated group 4 hours after vaccination, which can be classified as not clinically relevant. The highest individual increase in one vaccinated animal was 1.4 °C. In most animals, the temperature returned to baseline on day 1, in some animals on day 2 and some stayed straight above baseline during the two measured days. These findings are adequately reflected in section 4.6 of the SPC.

Local reactions were found in 90% of the vaccinated animals at 4 hours post-vaccination (86% mild, 4% moderate), which mostly disappeared until day 2 post vaccination. Some animals (4%) also showed a two-episode sequence already seen in the laboratory study. All local reactions completely disappeared by week 3 post vaccination without any treatment. These findings are adequately reflected in section 4.6 of the SPC.

The data show that the product is safe when used at a dose of 0.2 ml in pigs from three weeks of age.

Environmental risk assessment

An environmental risk assessment according to the 'Guideline for environmental risk assessment for immunological veterinary medicinal products' (EMEA/CVMP/074/95) was provided and the likelihood of hazard and resulting consequences can be considered negligible. Based on the phase I assessment, a study phase II environmental risk assessment is not considered necessary.

Mhyosphere PCV ID is expected to pose a negligible risk to the environment when used as recommended.

Overall conclusions on the safety documentation

The safety of Mhyosphere PCV ID was investigated in two placebo-controlled, randomised, blinded studies which are adequately described; one laboratory study and one multicentre field trial. In order to ensure maximum antigen content in the batch used in the laboratory study, it was agreed in a scientific advice (EMA/CVMP/SAWP/412022/2015) to further concentrate the antigenic fraction during production (without changing the proportion of the other components of the vaccine), which is considered as worst-case scenario. The field trial was conducted using two standard industrial batches.

In both studies, a sufficient number of animals of the most sensitive category (3-week-old piglets), the recommended route of administration (intradermal) and the recommended application scheme (one dose) were used to investigate the safety of the product. The studies are considered valid. Based on the results from these studies, it was concluded that the safety in target animals is acceptable when the vaccine is administered according to the recommended schedule and via the recommended route.

The post-vaccination increase in rectal temperature compared to the baseline is deemed slight, with a mean group increase of 0.28 °C and a maximum individual increase of 1.4 °C. In general, the temperature returned to baseline on day 1 (in some animals on day 2). These findings are adequately reflected in section 4.6 of the SPC and far below the Ph. Eur. requirements (mean group increase ≤ 1.5 °C, max. individual increase ≤ 2.0 °C).

The very common mild inflammations (< 3 cm) and the common moderate local reactions (3–5 cm) can have a two-episode sequence and completely disappear within approximately 3 weeks post-vaccination without treatment. These findings are adequately reflected in section 4.6 of the SPC.

No overdose study was carried out as this product is not a live vaccine.

Since the vaccination schedule consists of a single lifetime dose, no repeated dose study was conducted for this vaccine, which is acceptable.

Reproduction safety was not investigated. The applicant included a warning in SPC section 4.7 ('The use is not recommended during pregnancy and lactation').

The product is not expected to adversely affect the immune response of the target animals or of its progeny, and, therefore, no tests on the immunological functions were carried out.

A user safety assessment in line with the relevant guidance document has been presented. No hazard has been identified and the risk can be considered negligible, except the risk related to the mineral oil excipient included in the vaccine. For this ingredient, the standard warning was included in the product literature, which is acceptable.

No study of residues has been performed. All substances included in the composition of the vaccine are either listed in annex 1 of Commission Regulation (EU) No 37/2010 or in the list of substances

considered as not falling within the scope of Regulation (EC) No 470/2009. Consequently, a withdrawal period of zero days has been established. However, the excipient manganese sulfate monohydrate is included in annex 1 to Regulation (EU) No. 37/2010 for oral use only (Table 1, no MRL required). However, the current vaccine is destined to be administered via the intradermal route. According to EMEA/MRL/334/97, manganese is an essential element and a normal component of the diet in humans, and the use in veterinary medicinal products will add negligible amount to the manganese intake from its use as feed additive. The recommended dietary intake for pigs is 40 mg of manganese/kg feed. The applicant argued that the low amount of manganese sulphate applied once in a lifetime via intradermal route has no pharmacological activity and is therefore out of the scope of Regulations (EC) No 37/2010 and (EC) No 470/2009. Considering that manganese is an essential element being part of the normal diet of pigs and the low dose which is applied via intradermal route per dose (compared to the recommended oral dose of 40 mg), this approach is considered acceptable. The product is considered safe for the consumer.

No specific studies on interactions with other immunologicals or veterinary medicinal products were performed, and the standard warnings are included in the product literature, which is acceptable.

An assessment of the ecotoxicological hazards showed that the overall risk of the vaccine to the environment is effectively zero.

Part 4 – Efficacy

Introduction and general requirements

Mhyosphere PCV ID consists of the inactivated recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon, expressing the PCV2 capsid protein (CP). Thus, the vaccine is intended to protect against two swine pathogens at the same time. The vaccine is presented as emulsion for injection in which the concentrated antigenic fraction (aqueous phase) is adjuvanted with the oily phase (light mineral oil and surfactants); no preservative is included.
The final antigen concentration per dose (0.2 ml) is RP (relative potency) ≥ 1.3 for *M. hyopneumoniae* and RP ≥ 1.3 for PCV2 CP by comparison with reference vaccines. The route of administration is intradermal.
As demonstrated in two dose-response studies, the proposed minimum potency specification for *M. hyopneumoniae* and PCV2 CP is RP ≥ 1.3, respectively. The minimum protective dose for *M. hyopneumoniae* and PCV2 CP has been demonstrated at RP = 1.0. The batches used for demonstration of the respective minimum protective dose were established as reference vaccine batches for the control of the final product by means of ELISA potency determination.

The product is intended for intradermal administration use as a single dose of 0.2 ml to pigs from 3 weeks of age. Efficacy studies were carried out in piglets for fattening at 3 weeks of age, both under laboratory and field conditions. However, in three laboratory studies (both dose-response studies and one DOI *M. hyopneumoniae*) piglets of 4 weeks of age were used. In all efficacy trials, animals inoculated with PBS served as controls. A sufficient justification and discussion of the impact of the use of older animals in three out of eleven laboratory studies has been provided. Furthermore, the exact ages of the animals in all laboratory studies were presented.

According to the claims as currently proposed by the applicant, the vaccine is intended to reduce lung lesions associated with porcine enzootic pneumonia caused by *M. hyopneumoniae* and to reduce the incidence of these lesions (as observed in field studies). Furthermore, the vaccination is

intended to reduce viraemia, virus load in lungs and lymphoid tissues and the duration of the viraemic period associated with diseases caused by PCV2. Additionally, the reduction of culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases (as observed at 6 months of age in field studies) is claimed. Moreover, the applicant applied for the following claim "Efficacy against PCV2 genotypes a, b and d has been demonstrated in field studies."

The onset of immunity is claimed for *M. hyopneumoniae* as 3 weeks and for PCV2 as 2 weeks after intradermal vaccination. The duration of immunity is claimed as 23 weeks for *M. hyopneumoniae* and as 22 weeks for PCV2 after intradermal vaccination.

Furthermore, the proposed additional information was amended as follows: "In addition, a reduction in nasal and faecal shedding and the duration of nasal excretion of PCV2 was demonstrated in animals challenged at 4 weeks and at 22 weeks after vaccination."

The vaccine is intended to be administered to piglets for fattening, to cover the whole fattening period, especially during the susceptible period of infection between the decrease in maternal antibodies (weaning) and the onset of the acquired immunity in pigs.

Efficacy was demonstrated in compliance with Directive 2001/82/EC as amended, the Ph. Eur. chapter 5.2.7 and Ph. Eur. monograph 2448 'Porcine enzootic pneumonia vaccine (inactivated)'. There is no specific Ph. Eur. monograph for inactivated vaccines against PCV2 in pigs. In addition, the requirements as described in the Note for guidance EMEA/CVMP/852/99-FINAL 'Field trials with veterinary vaccines' were considered.

Scientific advice was given concerning queries on the potency of vaccine batches considered to evaluate the efficacy of the product (EMA/CVMP/SAWP/412022/2015). It has been agreed that the vaccine batches used in the efficacy studies have to be formulated to have the lower *M. hyopneumoniae* and/or PCV2 potency in order to justify the lower specification limits for potency of *M. hyopneumoniae* and PCV2. To comply with these CVMP requirements, pilot vaccine batches consisting of the same amount of adjuvants and excipients but with a different antigenic fraction concentration were manufactured at experimental scale and tested in the laboratory efficacy trials. Respective certificates of analysis of these pilot batches are provided in dossier section 4.B. Laboratory trials.

Scientific advice was also given concerning the challenge model. However, the applicant followed a different approach and provided sufficient justification and explanation for the deviation from the scientific advice upon request.

Challenge model

The *M. hyopneumoniae* challenge strain consists of a field strain isolated in Denmark in 1992 from the lungs of a pig suffering from enzootic pneumonia. The PCV2b challenge strain used was isolated in Spain in 2006 from post-weaning multisystemic wasting syndrome (PMWS) affected pigs. Further information on the *M. hyopneumoniae* challenge strain such as origin and history were provided as requested.

All animals were challenged intranasally with a single dose of *M. hyopneumoniae* using 5 ml (2.5 ml/nostril) per animal on three consecutive days which is in accordance with Ph. Eur. 2448.

The PCV2b challenge strain, was administered intranasally to animals as a single dose using 2-5 ml (1-2.5 ml/nostril) per pig.

Detailed information is provided on the development of the challenge models including the choice of challenge strains and the day of necropsy. Separate challenge infections were conducted in the laboratory efficacy trials, which is acceptable.

In summary, the challenge models developed for laboratory efficacy trials were adequately described (including the choice of challenge strains and the day of necropsy) and justified. The relevance of the challenge strains to the current epidemiological situation in the EU was satisfactorily discussed. An overall characterisation of both, *M. hyopneumoniae* and PCV2b challenge strains have been provided.

Efficacy parameters and tests

Challenge studies included vaccine groups and a corresponding placebo group inoculated with PBS, which served as unvaccinated control group. Animals were clinically monitored after vaccination and after the challenge infection. Severe diseased and moribund animals were euthanised for welfare reasons and pathological examinations were done and results were documented.

The primary efficacy variable to demonstrate efficacy of the product against *M. hyopneumoniae* disease was the evaluation of lung lesions. Post-mortem examinations were conducted on each pig after *M. hyopneumoniae* challenge to evaluate the extent of lung lesions. The percentage of affected lungs was calculated using the validated weighted scoring system, which is in accordance with the scoring system as described in Ph. Eur. monograph 2448. Additional clarification regarding details on pulmonary lesion scoring has been provided. Development of clinical signs and *M. hyopneumoniae* serum antibodies, *M. hyopneumoniae* tissue load in lungs, growth performance including mean body weight and average daily weight gain (ADWG) and culling rate, mortality and concomitant treatments were considered secondary variables.

The parameters chosen are considered appropriate for evaluating the efficacy of the product. The tests performed to evaluate *M. hyopneumoniae* tissue load in lungs and the antibody response were commercial qPCR and ELISA, respectively. Validation reports and standard operating procedures of methods (e.g. nested PCR, rtPCR) were satisfactorily provided. The scoring systems used for evaluation of clinical signs were in-house developed methods and can be accepted as covering a sufficiently broad range of clinical and pathological parameters to permit adequate assessment of infection with *M. hyopneumoniae*.

No specific Ph. Eur. monograph is available for PCV2 inactivated vaccines. The efficacy parameters selected to investigate efficacy against PCV2 were PCV2 viraemia (primary outcome), viral shedding (nasal and faecal), PCV2 virus load in lungs and lymphoid tissues, body weight gain, general clinical signs and PCV2 serum antibodies (secondary variables).

The parameters chosen are considered appropriate for evaluating the efficacy of the product. The tests performed to evaluate viraemia, viral shedding, tissue virus loads, and the antibody response were commercial qPCR and ELISA, respectively. The applicant clarified the method applied to calculate the duration of PCV2 viraemic period and PCV2 shedding period. Furthermore, a re-calculation of the duration of viraemia in days, from the day of challenge until the last sampling day, was provided with similar results. Furthermore, the validation report of the PCV2-specific quantitative PCR was provided. A proposal regarding the claims on PCV2 shedding via nasal fluids and faeces, as well as the duration of PCV2 shedding was provided and considered acceptable. The scoring systems used for evaluation of general clinical signs, were in-house developed methods and are acceptable as they cover a sufficiently broad range of clinical and pathological parameters to permit adequate assessment of infection with PCV2.

Efficacy documentation

The efficacy of Mhyosphere PCV ID was evaluated in eleven laboratory studies, six of which were carried out for *M. hyopneumoniae* and five for PCV2. All laboratory studies were conducted in accordance with GLP. One GCP-compliant clinical field trial was carried out to assess both safety and efficacy under field conditions. The efficacy laboratory studies were conducted for the proposed route and method of administration (intradermal), following vaccination of target animals (pigs for fattening) with the proposed minimum specifications of RP ≥ 1.3 for both *M. hyopneumoniae* and PCV2 CP.

All laboratory studies were well documented, and eight out of eleven studies were carried out in target animals of the youngest age category recommended for vaccination (3 weeks of age onwards) by means of a separate challenge infection against *M. hyopneumoniae* or PCV2. The applicant justified and discussed the impact of the use of older animals satisfactorily (*M. hyo* dose response, *M. hyo* DOI and PCV dose response). Furthermore, two additional studies (OOI and DOI) in animals of 3-weeks of age have been provided supporting an onset of immunity of 3 weeks and a duration of immunity of 23 weeks for *M. hyopneumoniae*. In addition, the exact ages of animals in all laboratory studies were provided. Sufficient information on the challenge model was provided. Pilot vaccine batches consisting of the same amount of adjuvant and excipients but with a different antigen fraction concentration per dose were manufactured according to the method proposed in Part 2 of the file and scientific advice (EMA/CVMP/SAWP/412022/2015). Two industrial standard production batches manufactured according to the method proposed in Part 2 of the file were used in the field trial. All study protocols including an addendum with the history of changes were provided.

Efficacy against porcine enzootic pneumonia (*Mycoplasma hyopneumoniae*)		
	Study for the determination of the antigen dose and the efficacy of Mhyosphere PCV ID vaccine against enzootic pneumonia in piglets.	
	Study of the onset of immunity of Mhyosphere PCV ID vaccine against enzootic pneumonia in piglets.	
	Study on the onset of immunity of 3 weeks of Mhyosphere PCV ID vaccine against enzootic pneumonia in piglets.	
	Study of the duration of immunity of Mhyosphere PCV ID vaccine against enzootic pneumonia in piglets.	
	Study of the duration of immunity of 161 days of Mhyosphere PCV ID vaccine against enzootic pneumonia	
	Study of the influence of maternally derived antibodies (MDA) against *M. hyopneumoniae* on Mhyosphere PCV ID vaccine efficacy in piglets.	
Efficacy against porcine circovirus Disease (PCVD; PCV2)		
	Study for the determination of the antigen	

	Mhyosphere PCV ID dose and the efficacy against porcine circovirus diseases (PCVD) in piglets.	
	Study of the duration of immunity of Mhyosphere PCV ID vaccine against porcine circovirus diseases (PCVD) in piglets.	
	Study of the onset of immunity of Mhyosphere PCV ID vaccine against porcine circovirus diseases (PCVD) in piglets.	
	Study of the efficacy of Mhyosphere PCV ID vaccine against porcine circovirus diseases (PCVD) in piglets.	
	Study of the influence of maternally derived antibodies (MDA) against PCV2 on Mhyosphere PCV ID vaccine efficacy in piglets.	
Field trial		
	Clinical evaluation of the efficacy and safety of Mhyosphere PCV ID.	

Laboratory trials

Dose determination

The proposed release specification of RP ≥ 1.3 for Mhyosphere PCV ID was established based on two dose-determinations studies; one for *M. hyopneumoniae* and one for PCV2.

Dose-response study for the determination of the antigen dose and the efficacy of the product against *M. hyopneumoniae* (porcine enzootic pneumonia) in piglets.

One study was carried out in piglets at approximately 4 weeks of age to establish the minimum protective/ effective dose of Mhyosphere PCV ID vaccine in terms of the *M. hyopneumoniae* potency against porcine enzootic pneumonia.

In this study, 3 groups of animals (pigs at 4 weeks of age) were used. Two different formulations of Mhyosphere PCV ID vaccine were tested, consisting of the same amount of adjuvant and excipients but with a different *M. hyopneumoniae* concentration in the antigenic fraction (high dose = RP = 1.84 and low dose = RP = 1.0). One vaccine dose with an RP of 1.84 for *M. hyopneumoniae* of a pilot batch was administered to group A. Another vaccine dose with an RP of 1.0 for *M. hyopneumoniae* of another pilot batch was administered to group B by the intradermal route on the right side of the neck. Group C remained unvaccinated and received PBS as a control by the intradermal route. All animals were challenged with a virulent *M. hyopneumoniae* challenge strain by intranasal instillation of 5 ml (2.5 ml per nostril) 34 to 36 days after vaccination. Following challenge, at the end of the study (days 56/57) animals were sacrificed and investigated for their percentage of affected lung tissue with *M. hyopneumoniae*-like lesions and severity of lung lesions (classified into three categories : 0 to 5%, > 5% to 10% and > 10% according to the percentage of affected lung lobe), seroconversion and clinical signs according to a guide to record and score

clinical signs.

Results: Vaccinated animals in groups A (high dose; 8.67%) and B (low dose; 9.02%) were found to have a significantly lower percentage of affected lungs (decrease of 50%) than non-vaccinated pigs (group C: 17.46%; Mann-Whitney test; $p<0.05$). Eighty-three per cent of the unvaccinated piglets showed a lung lesion score above 10% of affected lung lobe in comparison to 44% of the vaccinated piglets with high dose and 37% of the vaccinated piglets with low dose. No significant differences were observed for mean cumulative clinical score, mean clinical signs score or for the percentage of pigs with clinical signs after challenge at the end of the study (Mann-Whitney U test; $p>0.05$). All animals were negative for *M. hyopneumoniae* in nasal swab samples analysed by PCR before vaccination. All animals from the three groups were seronegative before vaccination. By study day 33 (one day before challenge), all vaccinated animals of Group B (low dose) and 94.4% of vaccinated animals of Group A (high dose) had detectable *M. hyopneumoniae* antibodies in serum samples. All animals of Group A (high dose) seroconverted (IRPC value > 35) 15 days after the challenge infection. In contrast, controls animals (Group C) remained seronegative until 8 days after challenge infection (5.3%); whereas 7 control animals (38.9%) seroconverted 21 days after challenge infection (day 55).

It was concluded that vaccination by the recommended route with doses above and below the minimum *M. hyopneumoniae* content in the antigenic fraction as recommended in the SPC (RP ≥ 1.3) was efficacious and met efficacy requirements 34 days post vaccination. The RP of 1.0 for the *M. hyopneumoniae* potency is qualified as minimum protective dose of the Mhyosphere PCV ID vaccine. The proposed minimum titre for the *M. hyopneumoniae* potency of RP ≥ 1.3 is demonstrated.

Dose-response Study for the determination of the antigen dose and the efficacy of the product against Porcine circovirus diseases (PCVD) in piglets.

One study was carried out in piglets at approximately 4 weeks of age to establish the minimum protective/ effective dose of Mhyosphere PCV ID vaccine in terms of the PCV2 potency against porcine circovirus diseases.

In this study, 4 groups of animals (pigs at 4 weeks of age) were used. Three different formulations of Mhyosphere PCV ID vaccine were tested, consisting of the same amount of adjuvant and excipients but with a different PCV2 CP concentration in the antigenic fraction (high dose: RP = 1.75; medium dose: RP = 1.22 and low dose: RP = 1.0). One vaccine dose with an RP of 1.75 for PCV2 CP of a pilot batch was administered to group A. Another vaccine dose with an RP of 1.22 for PCV2 CP of another pilot batch was administered to group B and a vaccine dose with an RP of 1.0 for PCV2 CP of another pilot batch was administered by the intradermal route at the right side of the neck. Group D remained unvaccinated and received PBS as a control by the intradermal route. All animals of all treatment groups were challenged intranasally with 2 ml (1 ml per nostril) with a virulent PCV2 genotype “b” challenge strain 28 days after vaccination. Following challenge on day 56 of the study animals were investigated for viraemia, PCV2 shedding in nasal fluids and faeces, viral loads in tissues (mesenteric lymph node, inguinal lymph node and tonsil, and lung), seroconversion and for clinical signs after challenge according to a guide to record and score clinical signs.

Results: Mean viral loads in serum of vaccinated groups (group A = high dose, group B = medium dose and C = low dose) were significantly lower at 14, 21 and 27 days post-challenge compared to non-vaccinated animals (group D = non-vaccinated) (Mann-Whitney U test; $p<0.05$). This was also the case for the mean area under the curve (AUC) between day -1 and day 27 post-challenge for the vaccinated groups compared to the unvaccinated control animals of group D. The proportion of viraemic piglets in vaccinated groups were significantly lower at 14, 21- and 27-days post-challenge

compared to control animals (χ2/Fisher test; $p<0.05$). In addition, the duration of viraemia from challenge infection until the end of the study was also significantly lower in vaccinated groups compared to the control group (Mann-Whitney U test; $p<0.05$). Viral loads in nasal secretion were also significantly lower in vaccinated animals on 14, 21 and 27 days after challenge infection compared to control animals (Mann-Whitney U test; $p<0.05$) as well as the mean AUC from day -1 until day 27 post-challenge (Mann-Whitney U test; $p<0.05$). The proportion of animals (nasally) shedding the virus was also significantly lower compared to control animals at 14, 21 and 27 days post challenge (χ2/Fisher test; $p<0.05$) as well as the duration of virus excretion via nasal secretions during the whole study (Mann-Whitney U test; $p<0.05$). The proportion of animals shedding the virus via faeces was also significantly lower at 14, 21 and 27 days post-challenge infection compared to control animals (χ2/Fisher test; $p<0.05$) as well as the duration of faecal virus excretion from challenge until the end of the study (Mann-Whitney U test; $p<0.05$). PCV2 viral loads in tissues were significantly lower in groups of vaccinated animals (Group A, B and C) compared to non-vaccinated animals (Group D) at the end of the study (day 28 post challenge; Student's t-test; $p<0.05$). As the infection model is subclinical, no statistically significant differences were observed for mean cumulative score, mean clinical signs score and the proportion of animals with clinical signs. In the vaccinated groups (A, B and C) seroconversion (S/P ratio > 0.5) was detected starting 7 days after the challenge infection. At 21 days after the challenge all vaccinated animals seroconverted, except for one animal of group C, which seroconverted on day 27 post challenge. The control animals receiving the placebo vaccination (group D) remained seronegative until 14 days after the challenge infection. PCV2 serum antibodies were detected from 21 days after challenge in 26.7% and at 27 days in 40% of the unvaccinated animals.

It was concluded that vaccination by the recommended route with doses above and below the minimum PCV2 CP content as recommended in the SPC (RP ≥ 1.3) was efficacious and met efficacy requirements 28 days post vaccination. An RP of 1.0 for the PCV2 CP potency is qualified as minimum protective dose of the Mhyosphere PCV ID vaccine. The proposed minimum titre for the PCV2 CP potency of RP ≥ 1.3 is demonstrated.

Additional study against PCV2 - Study of the efficacy of Mhyosphere PCV ID vaccine against porcine circovirus diseases (PCVD) in piglets.

One study was carried out in piglets at 3 weeks of age to investigate the efficacy of the minimum standard dose of Mhyosphere PCV ID vaccine in terms of the PCV2 potency against porcine circovirus diseases.

In this study, two groups of 15 animals of 3 weeks of age were used. A vaccine dose of 0.2 ml with an RP of 1.33 for PCV2 CP of a pilot batch was administered to group A by the intradermal route. Group B remained unvaccinated and received one dose of PBS by the intradermal route. Animals of the vaccinated group and of the unvaccinated group were challenged intranasal with 2 ml (1 ml per nostril) with virulent PCV2 genotype "b" challenge strain 28 days after vaccination. Following challenge, animals were investigated for viraemia, PCV2 shedding in nasal fluids and faeces, viral loads in tissues (mesenteric lymph node, inguinal lymph node and tonsil and lung), body weight gain, seroconversion and clinical signs according to a guide to record and score clinical signs.

Results: Mean viral loads in serum of vaccinated animals (Group A) were significantly lower at 15, 21- and 28-days post-challenge compared to non-vaccinated animals (Group B) (Mann-Whitney U test; $p<0.05$). This was also the case for mean AUC between day -1 and day 28 post-challenge for the vaccinated group compared to the unvaccinated control animals of group B. The proportion of viraemic piglets in the vaccinated group was significantly lower at 15, 21- and 28-days post-challenge compared to control animals (χ2/Fisher test; $p<0.05$). In addition, the duration of viraemia

from challenge infection until the end of the study was also significantly lower in vaccinated animals compared to the control group (Mann-Whitney U test; $p<0.05$). Viral loads in nasal secretions were also significantly lower in vaccinated animals on 15, 21 and 28 days after challenge infection compared to control animals (Mann-Whitney U test; $p<0.05$) as well as the mean AUC from day -1 until day 28 post-challenge (Mann-Whitney U test; $p<0.05$). The proportion of animals (nasally) shedding the virus was also significantly lower compared to control animals at 15, 21 and 28 days post challenge (day 15: group A: 2/14 versus group B: 13/14) ($\chi 2$/Fisher test; $p<0.05$) as well as the duration of virus excretion via nasal secretions during the whole study (Mann-Whitney U test; $p<0.05$). The proportion of animals shedding the virus via faeces was also significantly lower at 15, 21 and 28 days post-challenge infection compared to control animals ($\chi 2$/Fisher test; $p<0.05$) as well as the duration of faecal virus excretion from challenge until the end of the study (Mann-Whitney U test; $p<0.05$). PCV2 viral loads in tissues were significantly lower in groups of vaccinated animals (Group A) compared to non-vaccinated animals (Group B) at the end of the study (day 28 post challenge; Student's t-test; $p<0.05$). No statistically significant differences between vaccinated animals and non-vaccinated controls were observed, neither in mean body weight nor in ADWG. Again, as the infection model is subclinical, no statistically significant differences were observed for mean cumulative score, mean clinical signs score and the proportion of animals with clinical signs. After the experimental challenge infection seroconversion (S/P ratio > 0.5) started at 7 days post infection 64.2% and increased gradually. At 15 days after the challenge all vaccinated animals seroconverted, except for one animal, which remained seronegative until the end of the study. The control animals receiving the placebo vaccination (Group B) remained seronegative until 15 days after the challenge infection. PCV2 serum antibodies were detected from 21 days after challenge in 14.3% of the controls and at 28 days post challenge infection in 50% of the unvaccinated control animals.

It was concluded that vaccination by the recommended route with a standard minimum dose including an antigenic fraction content as recommended in the SPC (RP of 1.3 for PCV2 and RP of 1.3 for *M. hyopneumoniae*) was efficacious and met efficacy requirements 28 days post vaccination against PCV2b challenge infections.

Onset of immunity

Onset of immunity against *Mycoplasma hyopneumoniae* (Enzootic pneumonia)

Two studies were carried out in pigs at 3 weeks of age in compliance with Ph. Eur. requirements to investigate the onset of protection against *M. hyopneumoniae*. Animals were administered a single dose by the recommended intradermal administration route.

In the first study, groups of 20 animals of 3 weeks of age were used. A vaccine dose of 0.2 ml with an RP of 1.34 for *M. hyopneumoniae* of a pilot batch was administered to group A by the intradermal route on the right side of the neck. Group B stayed unvaccinated but received PBS instead of the vaccine by the intradermal route. All animals of both groups were challenged with virulent *M. hyopneumoniae* strain by intranasal instillation of 5 ml (2.5 ml per nostril) 27 to 29 days after vaccination. Following challenge, animals were investigated for their percentage of affected tissue with *M. hyopneumoniae*-like lesions and severity of lung lesions (classified into three categories: 0 to 5%, > 5 to 10% and > 10% according to the percentage of affected lung lobes), body weight gain, seroconversion, proportion of PCR positive animals in the lung and clinical signs according to a guide to record and score clinical signs.

Results: Vaccinated animals in group A were found to have a significantly lower percentage of affected lungs (decrease of 50%) than non-vaccinated pigs (group A: 8.32% and group B: 17.27%;

Welch test $p<0.05$). 65% of the unvaccinated piglets showed in comparison to 42% of the vaccinated piglets. The mean body weight was higher in vaccinated animals than in unvaccinated animals at the end of the study (Student's t-test $p<0.05$). The ADWG was also significantly higher in vaccinated animals compared to controls (day 26 to day 49; Student's t-test $p<0.05$). No significant differences were observed for mean cumulative clinical score, mean clinical signs score or for the percentage of pigs with clinical signs after challenge at the end of the study (Mann-Whitney U test $p>0.05$). All animals were negative for *M. hyopneumoniae* in nasal swab samples analysed by PCR before vaccination. All tissue samples from animals from both groups had 100% detectable *M. hyopneumoniae* by real-time PCR at necropsy. Thus confirming the presence of *M. hyopneumoniae* in all Mhyo-like lesions. All animals from both groups were seronegative before vaccination. By study day 26 (one day before challenge) all vaccinated animals had detectable *M. hyopneumoniae*-specific antibodies in serum samples in contrast to non-vaccinated animals which stayed seronegative until study day 48 (22 days after challenge infection).

It was concluded that vaccination by the recommended route with a standard minimum dose for *M. hyopneumoniae* as recommended in the SPC (RP of 1.3) was efficacious and met efficacy requirements against *M. hyopneumoniae* infections 27 days post vaccination. The onset of immunity was shown at 27 days (4 weeks) after vaccination.

A second study has been provided with the answers to the list of questions. In this study, groups of 24 (25) animals of 3 weeks of age were used. A vaccine dose of 0.2 ml with an RP of 1.35 for *M. hyopneumoniae* of a pilot batch was administered to group A by the intradermal route on the right side of the neck. Group B stayed unvaccinated but received PBS instead of the vaccine by the intradermal route. All animals of both groups were challenged with a virulent *M. hyopneumoniae* strain by intranasal instillation of 5 ml (2.5 ml per nostril) 21 to 23 days after vaccination. Investigation of animals following challenge was performed similar to the previous study (see above).

Results: Vaccinated animals in group A were found to have a significantly lower percentage of affected lungs than non-vaccinated pigs (group A: 8.26% and group B: 12.89%; Mann-Whitney U test $p<0.05$). 63% of the unvaccinated piglets showed more than 10% of affected lung in comparison to 39% of the vaccinated piglets. No significant differences could be observed for mean body weight or ADWG between vaccinated animals or control animals. No significant differences were observed for mean clinical signs score (Mann-Whitney U test $p>0.05$). All animals were negative for *M. hyopneumoniae* in nasal swab samples analysed by PCR before vaccination. The presence of *M. hyopneumoniae* was confirmed in lung tissue samples. All animals from both groups were seronegative before vaccination. By study day 21 (day before challenge) 37.5% of the vaccinated animals had detectable *M. hyopneumoniae*-specific antibodies in serum samples in contrast to non-vaccinated animals which stayed seronegative until study day 36 (15 days after challenge infection).

It was concluded that vaccination by the recommended route with a standard minimum dose for *M. hyopneumoniae* as recommended in the SPC (RP of 1.35) was efficacious and met efficacy requirements against *M. hyopneumoniae* infections 21 days post vaccination. Therefore, an onset of immunity is established at 21 days (3 weeks) after vaccination as stated in the SPC.

Onset of immunity against Porcine circovirus PCV2 (Porcine circovirus disease; PCVD)

One study was carried out in 3-week-old piglets in compliance with Ph. Eur. requirements to investigate the onset of protection. One dose of 0.2 ml was administered by the recommended intradermal route on the right side of the neck.

In this study, two groups of 24 animals of 3 weeks of age were used. A vaccine dose of 0.2 ml with an RP of 1.33 for PCV2 CP of a pilot batch was administered to group A by the intradermal route. Group B remained unvaccinated and received one dose of PBS by the intradermal route. Animals of the vaccinated group and of the unvaccinated group were challenged intranasally with 2 ml (1 ml per nostril) with a virulent PCV 2 genotype "b" challenge strain 2 weeks after vaccination. Following challenge, animals were investigated for viraemia, PCV2 shedding in nasal fluids and faeces, viral loads in tissues (mesenteric lymph node, inguinal lymph node and tonsil and lung) and for clinical signs after challenge according to a guide to record and score clinical signs.

Results: Most of the vaccinated animals in group A were found to have lower mean viral loads in serum on day 14 and 20 after challenge infection compared to control animals (Mann-Whitney U test $p<0.05$). This was also true for the mean AUC from challenge infection up to 27 days post-challenge, which was also significantly lower in vaccinated animals compared to controls. Furthermore, the proportion of viraemic piglets was significantly lower on days 14 and 20 in vaccinated animals than in piglets of the control group (day 14: 5/24 vs 16/23 and on day 20: 1/21 vs 8/22; $\chi 2$/Fisher test; $p<0.05$) as well as the duration of viraemia, which was significantly shorter in vaccinated animals (Mann-Whitney U test $p<0.05$). Vaccinated animals showed statistically significant lower mean viral loads in nasal fluids on days 14 and 27 post challenge infection compared to control animals (Mann-Whitney U test $p<0.05$). In addition, the mean AUC was also significantly lower in vaccinated animals between day 14 and day 27 post challenge. No significant differences were observed in the proportion of animals shedding the virus and the duration of virus excretion by nasal fluids. The proportion of animals shedding via faeces was found to be significantly lower for vaccinated animals on day 20 post-challenge compared to control animals (Mann-Whitney U test $p<0.05$). However, no significant difference was observed in this study regarding the duration of shedding via faeces between groups. Viral loads in mesenteric lymph node, tonsil and lung were significantly lower in vaccinated animals at the end of the study (day 28 after challenge) compared to control animals except for the inguinal lymph node (Mann-Whitney U test $p<0.05$). The applicant decided to maintain the onset of immunity of 2 weeks while deleting the general claim on virus shedding and duration of viral excretion. But, as virus shedding and the duration of viral excretion has been shown in all other studies provided, except for the OOI study, the applicant proposed to amend the SPC claims with additional information. This is acceptable when amended as follows: "In addition, a reduction in nasal and faecal shedding and the duration of nasal excretion of PCV2 was demonstrated in animals challenged at 4 weeks and at 22 weeks after vaccination." These proposed claims relating the shedding of PCV2 via faeces or nasal fluids and the duration of virus excretion were shown and are now in line with the results of all studies provided with the dossier. As the infection model is subclinical, there were no statistically significant differences in clinical signs after challenge infection between both groups (mean cumulative clinical score, mean clinical signs score and percentage of animals with clinical signs). All animals were seronegative or had low levels of maternally derived antibodies before vaccination. On the day of challenge vaccinated animals showed low antibody titres and seroconverted by day 14 after challenge infection in contrast to control animals in which 57.1% remained seronegative until day 27.

It was concluded that vaccination by the recommended route with a standard minimum dose for PCV2 CP as recommended in the SPC (RP of 1.3*)* was efficacious and met efficacy requirements 2 weeks post vaccination against PCV2b infections. The onset of immunity is established at 2 weeks after vaccination. No statistically significant reduction of the duration of virus excretion via nasal fluids and faeces have been shown for PCV2. Virus shedding was only significant on some days

tested (nasal fluids: day 14, day 27 and faeces: day 20). The onset of immunity of 2 weeks is maintained by the applicant and general claims were amended as outlined above. Minor amendments to the additional information regarding virus shedding and duration of viral excretion are introduced reflecting the findings in the studies provided appropriately.

Duration of immunity

Duration of immunity against *Mycoplasma hyopneumoniae* (Enzootic pneumonia)

Two studies were carried out in 4-week-old and 3-week old piglets to investigate the duration of immunity, by the recommended intradermal administration route.

In the first study, two groups of 24 pigs of 4 weeks of age were used. A vaccine dose of 0.2 ml from a pilot batch with an RP of 1.35 for *M. hyopneumoniae* was administered to group A by the intradermal route in the right side of the neck. Group B stayed unvaccinated but received PBS by intradermal administration as a control. All animals of both groups were challenged by intranasal route with 5 ml (2.5ml per nostril) of virulent *M. hyopneumoniae* strain (20 weeks after vaccination. Following challenge, animals were investigated for their percentage of affected tissue with *M. hyopneumoniae*-like lesions and severity of lung lesions (classified into three categories: 0 to 2, >2 to 5 and >5 according to the percentage of affected lung lobes), body weight gain, seroconversion, proportion of PCR positive animals in the lung and clinical signs according to a guide to record and score clinical signs.

Results: 22 days after challenge vaccinated animals in group A showed a significantly lower percentage of affected lungs (decrease of 50%) compared to controls (group A: 1.47% and group B: 3.04% affected lungs; Mann-Whitney U test $p<0.05$). 66.7% of the vaccinated piglets showed less than 2% of affected lung in contrast to only 28.6% of the non-vaccinated piglets. No statistically significant differences were observed in mean body weight or in ADWG between both groups (Student's t-test $p>0.05$). For mean clinical signs score, mean cumulative clinical score and the percentage of pigs with clinical signs after challenge no statistically significant differences between both groups were observed. *M. hyopneumoniae* tissue loads were significantly lower in vaccinated animals compared to non-vaccinated pigs (Mann-Whitney U test $p<0.05$). Although the percentage of positive lung samples was higher in non-vaccinated animals than in vaccinated animals (group A: 50% vs. group B: 81%) no statistically significant difference between groups could be observed ($\chi2$/Fisher test $p>0.05$). All animals from both groups were seronegative before vaccination. By study day 139 (one day before challenge) 25% of the vaccinated animals had detectable *M. hyopneumoniae*-specific antibodies in serum samples in contrast to non-vaccinated animals which stayed seronegative until the end of the study. 22 days after challenge infection, the percentage of seropositive animals in the vaccinated group was 54%.

Conclusion: In this study, 20 weeks post vaccination, which is the original claimed duration of immunity for the *M. hyopneumoniae*, a significant difference in the extent of lung lesions and the number of *M. hyopneumoniae* organisms in the lung was demonstrated between vaccinated groups and controls, sufficiently supporting the proposed duration of immunity. Duration of immunity was not investigated in MDA-positive animals.

A second study was provided with the answers to the list of questions. In this study, two groups of 22 pigs of 3 weeks of age were used. A vaccine dose of 0.2 ml from pilot a batch with an RP of 1.35 for *M. hyopneumoniae* was administered to group A by the intradermal route in the right side of the neck. Group B stayed unvaccinated but received PBS by intradermal administration as a control. All animals of both groups were challenged by intranasal route with 5 ml (2.5 ml per nostril) of a

virulent *M. hyopneumoniae* strain 23 weeks after vaccination on three consecutive days (day 161 to 163). Investigation of animals following challenge was performed similar to the previous study (see above).

Results: 22 days after challenge vaccinated animals in group A showed a significantly lower percentage of affected lungs compared to controls (group A: 1.96% and group B: 4.52% affected lungs; Welch test; $p<0.05$). 48% of the non-vaccinated piglets showed more than 5% of affected lung in contrast to only 5% of the vaccinated piglets. No statistically significant differences were observed in mean body weight or in ADWG between both groups (Student's t-test $p>0.05$). No significant differences were observed for mean clinical signs score, between both groups. *M. hyopneumoniae* tissue loads were significantly lower in vaccinated animals compared to non-vaccinated pigs (Student's t-test $p<0.05$). Although the percentage of positive lung samples was higher in non-vaccinated animals than in vaccinated animals (group A: 68% vs. group B: 76%) no statistically significant difference between groups could be observed ($\chi 2$/Fisher test $p>0.05$). All animals from both groups were seronegative before vaccination. By study day 81 100% of the vaccinated animals had detectable *M. hyopneumoniae*-specific antibodies in serum samples in contrast to non-vaccinated animals which stayed seronegative until day 175 (14 days post-challenge).

Conclusion: In this study a significant difference in the extent of lung lesions and the number of *M. hyopneumoniae* organisms in the lung was demonstrated between vaccinated groups and controls. Therefore, a duration of immunity of 23 weeks after vaccination is established and the SPC has been amended accordingly. Again, the duration of immunity was not investigated in MDA-positive animals.

Duration of immunity against Porcine circovirus PCV2 (Porcine circovirus disease; PCVD)

One study was carried out in 3-week-old piglets to investigate the duration of immunity by a single intradermal vaccination.

In this study, two groups of 14 piglets of 3 weeks of age were used. A vaccine dose of 0.2 ml from a pilot batch with an RP for PCV2 CP of 1.33 5) was administered to group A by the intradermal route in the right side of the neck. Group B stayed unvaccinated but received PBS by the intradermal route. Animals in both groups were challenged with a virulent PCV2 genotype "b" challenge strain with 4 ml (2 ml per nostril) 22 weeks after vaccination.

Results: Mean viral loads in serum of vaccinated animals were significantly lower on days 14 and 21 after challenge infection in vaccinated animals compared to control animals Mann-Whitney U test $p<0.05$). This was also true for the mean AUC from day 0 until day 27 post-challenge. The proportion of viraemic pigs on days 14, 21 and 27 were significantly lower in the vaccinated group compared to the control group $\chi 2$/Fisher test; $p<0.05$). Furthermore, the duration of viraemia was statistically significantly lower in vaccinated animals compared to control piglets (Mann-Whitney U test $p<0.05$). In vaccinated animals compared to control pigs mean viral loads in nasal secretions were significantly lower on days 14 and 27 post-challenge (Mann-Whitney U test $p<0.05$) as well as the mean AUC from day 0 until day 27 post challenge. The proportion of animals shedding the virus via nasal secretions was also significantly lower in vaccinated animals compared to controls on day 14 and day 27 after challenge infection ($\chi 2$/Fisher test; $p<0.05$). The duration of virus excretion by nasal fluids was also significantly lower in vaccinated animals (Mann-Whitney U test $p<0.05$). The proportion of animals shedding the virus via faeces was also significantly lower on day 14 post-challenge compared to control animals (Fisher test; $p<0.05$). The duration of virus excretion via faeces from challenge until the end of the study was not statistically significant (Mann-Whitney U

test p<0.05). Viral loads in all tissues investigated (lymphoid organs and lung) were significantly lower for all time points post-challenge (Mann-Whitney U test p<0.05). Again, as the challenge model was subclinical, no statistically significant differences were observed for mean cumulative clinical score, the mean clinical signs score and the percentage of pigs with clinical signs. Before vaccination all animals were either seronegative or had low levels of maternally derived antibodies. Before challenge infection, 5 out of 14 vaccinated pigs had detectable antibodies and all animals had a clear booster seroconversion by day 7 post challenge (71.4% seropositive), in contrast to control animals, which stayed seronegative until day 14 post-challenge infection (70% seronegative).

Conclusion: In this study 22 weeks post vaccination, which is the claimed duration of immunity against PCV2, significant difference in protection was demonstrated between vaccinated groups and controls, sufficiently supporting the proposed duration of immunity. Duration of immunity was not investigated in MDA-positive animals.

Maternally derived antibodies (MDA)

Maternally derived antibodies (MDA) against *M. hyopneumoniae*

One study was carried out in MDA-positive animals to investigate the efficacy of vaccination with a single dose of Mhyosphere PCV ID by the intradermal administration route.

In this study, 4 groups of animals (pigs at 3 weeks of age) were used. Three groups (groups A, B and C) included MDA positive animals. 2 of these 3 MDA positive groups had high levels of MDA (>65 IRPC in CIVTEST SUIS MHYO; group A and B, MDA++) and one group had medium levels of MDA (> 45 – 65 IRPC in CIVTEST SUIS MHYO; group C, MDA+). The remaining fourth group (< 35 IRPC in CIVTEST SUIS MHYO; group D, MDA-) was seronegative before vaccination. One vaccine dose of a pilot batch with an RP of 1.35 for *M. hyopneumoniae* was administered to groups A, C and D respectively by the intradermal route on the right side of the neck. Group B with high MDA (MDA++) stayed unvaccinated and received PBS by the intradermal route as a control. All animals were challenged with 5 ml of a virulent *M. hyopneumoniae* strain by the intranasal route (2.5 ml per nostril) 67 to 69 days after vaccination. At 22 to 23 days after challenge infection the percentage of affected lungs in all vaccinated groups (group A MDA++, group C MDA+ and group D MDA-) was significantly lower than in the unvaccinated group (group B, MDA++; Mann-Whitney U test p<0.05). No differences were observed between vaccinated groups. 61% of the unvaccinated piglets showed a lung lesion score above 10% in contrast to only 30% of the vaccinated piglets regardless of the MDA level at vaccination. No statistically significant differences were found for mean cumulative clinical score and mean clinical signs score. All animals were negative for *M. hyopneumoniae* in nasal swab samples analysed by PCR before vaccination. All tissue samples from animals from both groups had 100% detectable *M. hyopneumoniae* by real-time PCR at necropsy. At the end of the study almost all animals of the vaccinated groups had seroconverted (group A, MDA++: 89%, group C, MDA+: 94% and group D, MDA-: 100%) in contrast to non-vaccinated animals of group B (MDA++) which only showed 39% of seroconversion by the end of the study (day 88, 21 days after challenge infection).

It was concluded that vaccination by the recommended route with a standard minimum dose for *M. hyopneumoniae* as recommended in the SPC (RP of 1.3) was efficacious and met the Ph. Eur. 5.2.7 efficacy standard including MDA positive animals against *M. hyopneumoniae* experimental infections.

Maternally derived antibodies (MDA) against PCV2 (PCVD)

One study was carried out in MDA-positive animals to investigate the efficacy of vaccination with

Mhyosphere PCV ID by a single dose by the intradermal administration route.

In this study, 4 groups of animals (pigs, 3 weeks of age) were used. Three groups (groups A, B and C) included MDA positive animals. Two (2) of these 3 MDA positive groups had high levels of MDA (>1.4 S/P ratio in a PCV2 specific ELISA; group A and B, MDA++) and one group had medium levels of MDA (>0.8 to 1.4 S/P ratio in a PCV2 specific ELISA; group C, MDA+). The remaining fourth group (group D, MDA-) was seronegative before vaccination. One vaccine dose of a pilot batch with an RP of 1.33 for PCV2 (*M. hyopneumoniae* RP = 1.35) was administered to groups A, C and D respectively by the intradermal route on the right side of the neck. Group B with high MDA stayed unvaccinated and received PBS by the intradermal route as a control. All animals were challenged with a virulent PCV 2 genotype "b" - SP-107-54-13 isolate with 2 ml (1 ml per nostril) 56 days after vaccination. Mean viral loads in serum of vaccinated groups (group A = MDA++, group C = MDA+ and D = MDA-) were significantly lower at 14, 21 and 27 days post-challenge compared to non-vaccinated animals (group B = MDA++, Mann-Whitney U test $p<0.05$). This was also the case for mean AUC between day 0 and day 27 post-challenge for the vaccinated groups. The proportion of viraemic piglets in vaccinated groups were significantly lower at 14- and 27-days post-challenge compared to control animals ($\chi 2$/Fisher test; $p<0.05$). In addition, the duration of viraemia from challenge infection until the end of the study was also significantly lower in vaccinated groups compared to the control group (Mann-Whitney U test $p<0.05$). Viral loads in nasal secretion were also significantly lower in vaccinated animals 27 days after challenge compared to control animals (Mann-Whitney U test $p<0.05$) as well as the mean AUC from day 0 until day 27 post-challenge. The proportion of animals shedding the virus was also significantly lower compared to control animals at 27 days post challenge ($\chi 2$/Fisher test $p<0.05$) as well as the duration of virus excretion via nasal secretions during the whole study (Mann-Whitney U test $p<0.05$). The proportion of animals shedding the virus via faeces was also significantly lower at 21 days post-challenge infection compared to control animals ($\chi 2$/Fisher test $p<0.05$) as well as the duration of virus excretion from challenge until the end of the study (Mann-Whitney U test $p<0.05$). Mean PCV2 viral loads in tissues investigated were significantly lower in groups of vaccinated animals compared to non-vaccinated animals at the end of the study (day 28 post challenge; Student's t-test $p<0.05$). Again, as the challenge was sub-clinical, no statistically significant differences were observed for mean cumulative score, mean clinical signs score and the percentage of animals with clinical signs. All animals (100%) of group A and C (MDA++ and MDA+) seroconverted (S/P ration>0.05) 14 days after challenge infection and animals of group D (MDA-) 21 days post-challenge infection in contrast to non-vaccinated animals which only showed 66.7% of seroconversion by the end of the study (27 days post-challenge).

It was concluded that vaccination by the recommended route with a standard minimum dose for PCV2 as recommended in the SPC (RP of 1.3) was efficacious and met the Ph. Eur. 5.2.7 efficacy standard including MDA positive animals against PCV2b experimental infections. The classification of the MDA titres in three categories has been sufficiently clarified.

Interactions

No studies investigating the efficacy of Mhyosphere PCV ID when administered together with other veterinary medicinal products at the same time have been submitted. Therefore, the proposed standard wording of SPC section 4.8 is regarded as adequate.

Field trials

Clinical evaluation of the efficacy and safety of Mhyosphere PCV ID

One multicentre, randomised, double-blinded, placebo-controlled and GCP-compliant clinical field trial, was carried out to assess both the safety and efficacy of Mhyosphere PCV ID in controlling *M. hyopneumoniae* and PCV2 infections in clinically healthy fattening piglets under field conditions. The study was conducted on seven commercial pig farms (farrowing, nursery and fattening) located in France (five farms) and Hungary (two farms) with historical records of clinical or subclinical *M. hyopneumoniae* and/or PCV2 related disease with recently confirmed presence of at least one of the two pathogens. The field study started in April 2018 and finished in January 2019. Animals were randomly allocated to two treatment groups balanced by sow and body weight. Animals of both groups were administered either the vaccine Mhyosphere PCV ID or a placebo (PBS). The vaccination schedule consisted of a single dose of 0.2 ml administered intradermally on the right side of the neck at 3 weeks of age.

For statistical analysis of the efficacy study variables, three population datasets were established to perform the baseline analysis. The "Biological sampling population dataset" included animals selected for blood and swab sampling in each farm. A total of 428 pigs (213 in the placebo group and 215 in the vaccine group) of the total number of 2,507 enrolled animals were included in this dataset. The "Lung lesion population dataset" included 2,208 lungs (1,103 in the Placebo group and 1,106 in the vaccine group) and correspond to those animals sent to slaughterhouse and whose lungs could be evaluated. The "Weight population dataset" included the animals selected for weighing in each farm. A total of 1,381 pigs (690 in the placebo group and 691 in the vaccine group) of the total number of 2,507 animals enrolled for this study were included. Statistical analyses were performed including farm as a random factor in the appropriate statistical model.

Animals previously vaccinated against *M. hyopneumoniae* and/or PCV2 were excluded from the study. Furthermore, animals suffering a severe infectious process during their lifetime, such as intense diarrhoeas or pneumonias, as well as apparently healthy animals sharing a pen with animals with clinical signs compatible with an infectious disease at day 0, and also animals with abnormal physical conditions, such as lameness or severe hernia, were not included in this field study.

The study was well designed and conducted. The applicant used a sufficient number of animals and the recommended dose (0.2 ml), administration route (intradermal) and application scheme to investigate the efficacy of the product under field conditions. Two industrial standard batches were administered.

The efficacy parameters were evaluated between the day of vaccination until the end of the fattening period (6 months). The primary efficacy variable for *M. hyopneumoniae* infection is the incidence of *M. hyopneumoniae* associated pulmonary lesions at slaughter, whereas the primary efficacy variable for PCV2 infection is PCV2 viraemia. Secondary variables include the serological response to both *M. hyopneumoniae* and PCV2 infection, PCV2 nasal and faecal shedding, antibiotic treatments against respiratory and digestive diseases, ADWG between the vaccination until the end of fattening period and mortality rate.

Results:

M. hyopneumoniae: Reduction of lung lesions caused by *M. hyopneumoniae* could be observed in each tested commercial pig farm. Analysis of *M. hyopneumoniae* lung lesions at slaughter showed a significant reduction in the percentage of affected lung surface in vaccinated pigs compared to control pigs ($p < 0.05$; Mann-Whitney Test). The mean lung lesion score in the vaccinated group was significantly lower than in the placebo group ($p < 0.05$; Mann-Whitney Test). Significant differences were observed in the severity of lung lesions, whereas vaccinated animals (35%) showed a lung lesion percentage above 10% compared to control animals (45%) ($p < 0.05$; Chi square Test). The incidence of pleurisy (indication of secondary infections with pathogens) or fissures/scarring

(resolved lesions attributed to old *M. hyopneumoniae* infections in the pig herd) was lower in the group vaccinated with Mhyosphere PCV ID than in the placebo group but no statistically significant or clinically relevant differences were observed between groups with respect to the incidence of lungs with pleurisy and fissures. 60% of all animals from both groups were seropositive (IRPC value > 35) against *M. hyopneumoniae* before vaccination. The rate of seropositive animals slowly decreased up to week 8 after vaccination. After that, the antibody levels gradually increased. The percentage of seropositive animals at each time point shows that on week 8, week 12, week 19 and week 22 after vaccination a statistically significant higher percentage of animals from the vaccinated group were seropositive compared to the placebo group ($p < 0.05$; generalised mixed model with a binomial response considering "Farm" as a random effect).

PCV2: PCV2 viraemia, measured by PCV2 virus load in serum (qPCR), was significantly lower in vaccinated animals compared to non-vaccinated animals during the whole study period. This was also the case for the mean AUC between day 0 and 22 weeks after vaccination for the vaccinated group compared to the unvaccinated control animals of group A ($p < 0.05$; linear mixed model considering "Farm" as a random effect). The mean PCV2 viraemia at each time point was significantly lower in the vaccinated group than in the unvaccinated control group between week 8 after vaccination until the end of the study (week 22 after vaccination) ($p < 0.05$; linear mixed model considering "Farm" as a random effect). However, the PCV2 circulation in each farm was detected at different time points and needs to be taken into account by reviewing these data. Analysis of the proportion of viraemic piglets (serum PCV2 virus load) at each time point revealed a significantly lower percentage of animals of the vaccinated group positive to qPCR when compared to the unvaccinated control group from 10 weeks after vaccination onwards ($p < 0.05$; linear mixed model considering "Farm" as a random effect).

Viral loads in nasal swabs measured by qPCR were significantly lower in the vaccinated animals from week 10 to week 22 after vaccination compared to control animals as well as the mean AUC from week 0 until week 22 after vaccination ($p < 0.05$; linear mixed model considering "Farm" as a random effect). PCV2 nasal shedding at each time point revealed that vaccinated animals showed a significant reduction in the mean PCV2 nasal shedding starting 10 weeks after vaccination until week 22 after vaccination ($p<0.05$; linear mixed model considering "Farm" as a random effect). The proportion of animals (nasally) shedding the virus was also significantly lower compared to control animals at 19 and 22 weeks after the vaccination (week 19: group B: 66.7% versus group A: 72.9%, $p<0.05$; generalised mixed model with a binomial response considering "Farm" as a random effect). Between week 6 and week 22 after vaccination mean viral loads in faecal excretions were also significantly lower in vaccinated animals compared to controls as well as the mean AUC from week 6 until week 22 after vaccination ($p<0.05$; linear mixed model considering "Farm" as a random effect). PCV2 faecal shedding at each time point revealed that vaccinated animals showed a significant reduction in the mean PCV2 faecal shedding starting 8 weeks after vaccination until week 22 after vaccination ($p<0.05$; linear mixed model considering "Farm" as a random effect). The proportion of animals shedding the virus via faeces was also significantly lower compared to control animals at 19 and 22 weeks after the vaccination (generalised mixed model with a binomial response considering "Farm" as a random effect). 86-89% of all animals from both groups were seropositive (S/P ratio > 0.5) against PCV2 before vaccination. The rate of seropositive animals slowly decreased 3 weeks after vaccination in both groups. The percentage of seropositive animals at each time point shows that on week 8, week 14 and week 19 after vaccination a statistically significant higher percentage of animals from the vaccinated group were seropositive compared to the placebo group (generalised mixed model with a binomial response variable considering "Farm" as a random effect). On week 22 after vaccination, differences in the percentage of seropositive animals were no longer observed.

Further data as well as a validation study were submitted in relation to PCV2 genotype analysis of circulating PCV2 viruses on each pig farm and support the proposed SPC claim: "*Efficacy against PCV2 genotypes a, b and d has been demonstrated in field studies*".

Analysis of the growth performance of the pigs demonstrated a significant reduction (54%) of the culling rate (percentage of animals < 75 kg) at the end of the fattening period in the group vaccinated with Mhyosphere PCV ID compared to the placebo group (p<0.05; Chi square Test). Regarding the mean body weight, vaccinated animals weighed 2.61 kg more than control animals at the end of the fattening period (p<0.05; linear mixed model considering "Farm" as a random effect). No significant differences were observed in the ADWG between the two treatment groups during the post-weaning period from 3 to 9 weeks of age. During the fattening period, from 9 weeks of age until slaughter, the ADWG of vaccinated animals was significantly higher than that of the control group (p<0.05; linear mixed model considering "Farm" as a random effect). When considering the overall study period, from weaning until end of fattening, the ADWG of vaccinated animals was significantly higher than that of the control animals (p<0.05; linear mixed model considering "Farm" as a random effect). The overall result of vaccinated pigs showed a significant increase of 17.61 g weight gain per day compared to control animals (p<0.05; linear mixed model considering "Farm" as a random effect).

There were no statistically significant differences between the group vaccinated with Mhyosphere PCV ID and the placebo group in terms of mortality and considering concomitant treatment.

In conclusion, the data of this field trial showed that the product is efficacious to reduce the incidence of lung lesions associated with porcine enzootic pneumonia caused by *M. hyopneumoniae* at an intradermal dose of 0.2 ml in 3-week-old piglets for fattening. The results, which were only observed in field trials were adequately reflected in the SPC. The product is also efficacious to reduce viraemia, the percentage of viraemic pigs, PCV2 nasal and faecal shedding and the percentage of PCV2 shedder pigs (nasal and faecal) associated with PCV2 related diseases as observed under field conditions. In addition, the product reduced the culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases at 6 months of age as observed in this field trial. A clarification regarding the divergent numbers stated for the selection of animals in the population dataset for PCV2 detection has been provided.

Overall conclusion on efficacy

The minimum standard dose of inactivated recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon was established based on two dose-finding studies with doses above and below the antigen content as recommended in the SPC [RP ≥ 1.3; RP (ELISA tests) for both *M. hyopneumoniae* and PCV2 CP by comparison with reference vaccines].
For *M. hyopneumoniae,* Mhyosphere PCV ID vaccine batches above and below the minimum content as recommended in the SPC (RP ≥ 1.3) were applied to 4-week-old piglets (*M. hyopneumoniae* RP = 1.84 and RP = 1.0). All vaccine batches under test met efficacy requirements after intranasal virulent *M. hyopneumoniae* challenge infection 34 days post vaccination. An RP of 1.0 for the *M. hyopneumoniae* potency is qualified as minimum protective dose of the vaccine. This batch was established as reference vaccine batch for batch potency testing. To take the inherent variability of the test into account, the release limit was established by using the upper limit of the 95% confidence interval resulting in an RP of 1.3. Thus, the proposed minimum specification for the *M. hyopneumoniae* potency of RP ≥ 1.3 as stated in the SPC has been adequately justified.
For PCV2, Mhyosphere PCV ID vaccine batches above and below the minimum content as recommended in the SPC (RP ≥ 1.3) were applied to 4-week-old piglets (PCV2 CP RP = 1.75, RP = 1.22 and RP = 1.0). All vaccine batches under test met efficacy requirements after intranasal virulent

PCV2b challenge infection 28 days post vaccination. An RP of 1.0 for the PCV2 CP potency is qualified as minimum protective dose of the vaccine analogous to *M. hyopneumoniae*. This batch was established as reference vaccine batch for batch potency testing. To take the inherent variability of the test into account, the release limit was established by using the upper limit of the 95% resulting in an RP of 1.3. Thus, the proposed minimum specification for the PCV2 CP potency of RP ≥ 1.3 as stated in the SPC has been adequately justified.
In an additional laboratory study, a vaccine batch with a standard minimum dose for the antigenic fraction as recommended in the SPC (PCV2 CP RP = 1.33) applied to 3-week-old piglets met efficacy requirements 28 days post vaccination against an intranasal virulent PCV2b challenge infection. This study supports the proposed minimum standard titre for PCV2 CP potency of RP ≥ 1.3 additionally.

A justification of the impact of the use of older animals in three out of eleven laboratory studies has been provided for *M. hyopneumoniae* as well as for PCV2. Two additional studies were provided with the responses to the list of questions. One onset of immunity and one duration of immunity studies performed in 3-week-old piglets were presented. Challenge infections were performed 3 weeks and 23 weeks after vaccination. Consequently, an onset of immunity of 3 weeks and a duration of immunity of 23 weeks has been satisfactorily established for *M. hyopneumoniae*.

The results from three laboratory trials demonstrate a consistent significant reduction in the percentage of affected lung, but no significant reduction in the percentage of pigs showing more than 10% of affected lung surface was shown. Based on a clarification the claim regarding the incidence of lung lesions was amended with the wording “as observed in field trials” whereas the claim “severity of lesion” has been deleted. One field trial showed that the product is effective to reduce incidence and severity of lung lesions associated with porcine enzootic pneumonia caused by *M. hyopneumoniae.* Clarification regarding the pulmonary lesion scoring has been satisfactorily provided. Results from the field study were adequately reflected in the SPC. The product is also considered to be effective in reducing the culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases as observed until slaughter in field studies. The proposed indication for efficacy against PCV2 genotypes a, b and d has been sufficiently supported by additional data provided.

Onset and duration of immunity were similar with a vaccine batch at a minimum RP of 1.3 or higher for both *M. hyopneumoniae* and PCV2. Results supported a single vaccination at 3 weeks of age with a minimum RP of 1.3 or higher per dose. The product has been shown to have an onset of immunity 3 weeks after vaccination for *M. hyopneumoniae*.

The applicant amended the SPC wording regarding the duration of virus excretion and virus shedding as follows “to reduce viraemia, virus load in lungs and lymphoid tissues, and the duration of the viraemic period associated with diseases caused by Porcine circovirus type 2 (PCV2) associated diseases. Efficacy against PCV2 genotypes a, b and d has been demonstrated in field studies. The applicant maintained the onset of immunity of 2 weeks for PCV2. Following additional information section 4.2 of the SPC has been amended as follows: “In addition, a reduction in nasal and faecal shedding and the duration of nasal excretion of PCV2 was demonstrated in animals challenged at 4 weeks and at 22 weeks after vaccination.” Duration of immunity of 23 weeks against *M. hyopneumoniae* infections and of 22 weeks against infections with PCV2 have been shown adequately.

MDA against *M. hyopneumoniae* or PCV2 did not interfere with vaccination.

One field study was undertaken in seven commercial pig farms for fattening with historical records of clinical or subclinical PCV2 and/or *M. hyopneumoniae* related diseases and with recently confirmed presence of at least one of the two pathogens. Results of this field study confirmed all

claims stated in the SPC for porcine enzootic pneumonia caused by *M. hyopneumoniae*. For PCV2 related diseases a reduction of viraemia, the proportion of viraemic pigs, virus shedding (nasal and faecal) and the proportion of pigs shedding the virus has been shown. In addition, the vaccine did support the reduction of the culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases. Relevant information concerning the digestive outbreaks as well as a summary of *M. hyopneumoniae* results per farm were provided. The wording regarding the culling rate and daily weight gain has been adequately reflected in the SPC as follows: "as observed at 6 months of age in field studies". Further data of the field study provided support the efficacy of the vaccine against PCV2 genotypes a, b and d.

Overall, according to the data collected in the laboratory and field efficacy trials, it can be concluded that Mhyosphere PCV ID is an efficacious vaccine against porcine enzootic pneumonia caused by *M. hyopneumoniae* and related diseases, and also efficacious against PCV disease caused by PCV2 and related diseases. The PCV claim regarding the reduction of viraemia, virus load in lungs and lymphoid tissues and the duration of the viraemic period associated with PCV2 related diseases has been sufficiently shown. The claim regarding virus shedding and the duration of virus excretion for PCV2 was amended and reflects now the findings in all pivotal PCV2 efficacy studies appropriately.

The duration of immunity and the efficacy of vaccination have been adequately shown under laboratory and field conditions.

The proposed onset of immunity of 2 weeks for PCV2 is acceptable with the revised general claims. Additional information proposed by the applicant under section 4.2 of the SPC regarding virus shedding and duration of viral excretion was amended and is therefore in line with the results of all studies provided.

Part 5 – Benefit-risk assessment

Introduction

Mhyosphere PCV ID consists of the inactivated recombinant *M. hyopneumoniae*^{cpPCV2}, strain Nexhyon, expressing the PCV2 capsid protein (CP). Thus, the vaccine is intended to protect against two swine pathogens at the same time. The antigenic fraction is adjuvanted with light mineral oil. No preservative is added. The pharmaceutical form of the final vaccine is an emulsion for injection. Mhyosphere PCV ID is innovative because the recombinant vaccine strain Nexhyon induces immunity against two relevant swine pathogens, *M. hyopneumoniae* and PCV2 at the same time, which are frequently isolated as co-infectors in growing and finishing pigs.

The vaccine is intended for the active immunisation of pigs to reduce lung lesions associated with porcine enzootic pneumonia caused by *Mycoplasma hyopneumoniae* and to reduce the incidence of these lesions (as observed in field studies). Furthermore, the vaccination is intended to reduce viraemia, virus load in lungs and lymphoid tissues and the duration of the viraemic period associated with diseases caused by Porcine circovirus type 2 (PCV2). Efficacy against PCV2 genotypes a, b and d has been demonstrated in field studies.

Additionally, the reduction of culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases (as observed at 6 months of age in field studies) is claimed.

Clarification regarding the claims of severity and incidence of lung lesions has been provided.

In addition, the applicant proposes the following wording as additional information "A reduction in

PCV2 virus shedding and in the duration of the viral excretion have been demonstrated from 4 weeks up to 22 weeks after vaccination and in field studies". This is also in accordance with EMEA/CVMP/042/97 'Revised position paper on indications for veterinary vaccines' and with the Notice to Applicants Volume 6C which allows further information on the protection that is supported by valid trial data.
As in all studies, except for the OOI study, a reduction in virus shedding and a reduction in the duration of nasal excretion has been shown, this additional claim is acceptable. The duration of virus excretion for PCV2 via faeces has been significantly reduced in most studies provided, but results were not significant in the OOI and DOI study. The claim proposed by the applicant was amended and reflect the results now appropriately.

The wording of this additional information was amended as follows: "In addition, a reduction in nasal and faecal shedding and the duration of nasal excretion of PCV2 was demonstrated in animals challenged at 4 weeks and at 22 weeks after vaccination."

The proposed dose of 0.2 ml, the route of administration (intradermal) and the vaccination scheme (one dose to pigs from 3 weeks of age onwards) have been confirmed.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC (full application).

Benefit assessment

Direct therapeutic benefit

The benefit of Mhyosphere PCV ID is its efficacy in vaccinated pigs to reduce lung lesions associated with porcine enzootic pneumonia caused by *M. hyopneumoniae*. Regarding PCV2 a reduction of viraemia, virus load in lungs and lymphoid tissues, and the duration of the viraemic period associated with PCV2 related diseases have been sufficiently shown. Claims regarding the reduction in virus shedding and the duration of viral excretion were further amended reflecting now the findings of the studies provided appropriately. Studies were performed in a large number of well designed, controlled laboratory challenge studies conducted in accordance with GLP. In addition, reduction of culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases as well as the incidence of lung lesions caused by *M. hyopneumoniae* were demonstrated in the field study which was conducted in accordance with GCP. The beneficial effects persisted until 6 months of age in field studies.

The proposed claim for particular PCV2 genotypes is considered satisfactorily supported.

The proposed onset of immunity of 3 weeks and the proposed duration of immunity of 23 weeks for *M. hyopneumoniae* after intradermal vaccination are supported. The proposed onset of immunity of 2 weeks for PCV2 and the proposed duration of immunity of 22 weeks for PCV2 after intradermal vaccination is substantiated by appropriate data.

Additional benefits

Mhyosphere PCV ID is easy to apply to young piglets by single intradermal vaccination using an appropriate device.

One single vaccination is sufficient to stimulate immunity against two relevant swine pathogens, *M. hyopneumoniae* and PCV2, for the complete fattening period (6 months).

Mhyosphere PCV ID increases the range of available treatment possibilities (prophylaxis possibilities) for the active immunisation of pigs against porcine enzootic pneumonia and PCV2 related diseases.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics.

Safety:

Risks for the target animal:

Administration of Mhyosphere PCV ID in accordance with SPC recommendations is generally well tolerated. The main reported adverse reactions include local reactions at the administration site and slight transient temperature increase. The very common mild inflammations (<3 cm) and common moderate local reactions (3-5 cm), which can have a two-episode sequence, completely disappear within approximately 3 weeks post-vaccination without any treatment. These findings are adequately reflected in section 4.6 of the SPC. The transient post-vaccination increase in rectal temperature compared to the baseline is deemed slight with a mean group increase of 0.28 °C and a maximum individual increase of 1.4 °C. In most cases, the temperature returned to baseline values after one day, in some animals after two days, which is reflected in the SPC adequately.

Risk for the user:

It is concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice for products containing mineral oil is included in the SPC.

Risk for the environment:

Mhyosphere PCV ID is not expected to pose a risk for the environment when used according to the SPC recommendations.

Risk for the consumer:

All substances included in the composition of the vaccine are listed in Annex 1 of Commission Regulation (EU) 37/2010 or in the list of substances considered as not falling within the scope of Council Regulation (EEC) No 470/2009 and a withdrawal period of zero days is proposed. However, the excipient manganese sulphate monohydrate is included in Regulation (EU) No. 37/2010 for oral use only (Table 1, no MRL required). But the current vaccine is administered via intradermal route. Therefore, the applicant argued that the amount of manganese sulphate applied via intradermal route has no pharmacological activity and is therefore out of the scope of Regulations 27/2010 and 470/2009. This approach is considered acceptable.

Special risks:

Mhyosphere PCV ID is not expected to pose a special risk.

Risk management or mitigation measures

No specific risks from use of this product were identified and consequentially no specific risk management or mitigation measures are proposed.

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

Evaluation of the benefit-risk balance

The product has been shown to have a positive benefit-risk balance overall. The product has been shown to be efficacious for the active immunisation of pigs to reduce lung lesions caused by *M. hyopneumoniae* infections. Clarification on lung lesion scoring has been provided. The claim regarding the incidence of lung lesions caused by *M. hyopneumoniae* was amended with the wording "as observed in field studies" which is acceptable. The claim regarding the severity was deleted. Furthermore, the product has been shown to be efficacious for the active immunisation of pigs to reduce viraemia, virus load in lungs and lymphoid tissues, and the duration of the viraemic period associated with PCV2 related diseases. The results of all pivotal PCV efficacy studies are now adequately described; the claims regarding virus shedding and duration of viral excretion were amended to reflect the findings in the studies provided.

The claim regarding the reduction of the culling rate and the loss of daily weight gain caused by *M. hyopneumoniae* and/or PCV2 related diseases (as observed at 6 months of age in field studies) has been satisfactorily amended.

The claim regarding the differentiation between the PCV2 genotypes a, b and d demonstrated in field studies has been satisfactorily supported.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. The vaccine is well tolerated by the target animals and presents a low risk for users and the environment and appropriate warnings have been included in the SPC. A withdrawal period of zero days has been set.

Conclusion[1]

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 Mhyosphere PCV ID 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 by consensus that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned veterinary medicinal product.