10 September 2015
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Veterinary Medicines Division

## Committee for Medicinal Products for Veterinary Use (CVMP)

# CVMP assessment report for Suvaxyn Circo+MH RTU (EMEA/V/C/003924/0000)

Common name: Porcine circovirus and porcine enzootic pneumonia vaccine (inactivated)

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

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# Introduction

On 26 September 2014, the applicant Zoetis Belgium SA submitted an application for a marketing authorisation to the European Medicines Agency (the Agency) for Suvaxyn Circo+MH RTU under Article 3(1) of Regulation (EC) No. 726/2004 (biotechnological veterinary medicinal product).

The eligibility to the centralised procedure was agreed upon by the CVMP on 19 December 2013 as the product is developed by means of a biotechnological process. The rapporteur appointed was B. Urbain and the co-rapporteur was E.-M. Vestergaard.

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

Suvaxyn Circo+MH RTU is an emulsion for injection for pigs containing an inactivated *Mycoplasma (M.) hyopneumoniae* antigen strain P-5722-3 and inactivated recombinant porcine circovirus type 1 expressing the porcine circovirus type 2 ORF2 protein (cPCV1-2).

The proposed indication is for the active immunisation of finishing pigs against porcine circovirus type 2 (PCV2), to reduce viral load in blood and lymphoid tissues, faecal shedding caused by infection with PCV2, and against *M. hyopneumoniae* to reduce lung lesions caused by infection with *M. hyopneumoniae.* The proposed target species is pigs (for fattening) from 3 weeks of age. The proposed route of administration is intramuscular.

Suvaxyn Circo+MH RTU is presented in a cardboard box containing 1 high density polyethylene (HDPE) vial of 50 ml of product which is equivalent to 25 doses or 100 ml of product equivalent to 50 doses or 250 ml equivalent to 125 doses.

On 10 September 2015, the CVMP adopted an opinion and CVMP assessment report.

On 6 November 2015, the European Commission adopted a Commission Decision granting the marketing authorisation for Suvaxyn Circo+MH RTU.

## *Scientific advice*

Not applicable.

## *MUMS limited market status*

Not applicable.

# Part 1 – Administrative particulars

## *Detailed description of the pharmacovigilance system*

A detailed description of the pharmacovigilance system (version 1.2 dated 18/07/2013), which fulfils the requirements of Directive 2001/82/EC, was provided. Based on the information provided the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the European Union (EU) or in a third country are in place.

## *Manufacturing authorisations and inspection status*

**Manufacturer of the active substances**

Production of porcine circovirus type 1–type 2 (cPCV1-2) antigen, including in-process testing (excluding sterility testing on antigen fluids before inactivation and on inactivated antigen stock after neutralisation) is performed by Zoetis Inc., Charles City, Iowa (US).

Production of *M. hyopneumoniae* antigen, including in-process testing, is conducted by Zoetis LLC, Lincoln, Nebraska (US). Sterility testing on cPCV1-2 antigen fluids before inactivation and on cPCV1-2 inactivated antigen stock after neutralisation, is also conducted by Zoetis LLC, Lincoln, Nebraska (US).

Valid certificates of Good Manufacturing Practice (GMP) compliance, issued by the UK authorities, are provided for Zoetis Inc., Charles City, Iowa and for Zoetis LLC, Lincoln, Nebraska.

**Manufacturer responsible for batch release**

Secondary packaging and batch release for the EU is carried out by Zoetis Belgium SA, Louvain-La-Neuve, Belgium.

A valid manufacturing authorisation and certificate of GMP compliance issued by the Belgian authorities are presented for Zoetis Belgium SA, Louvain-La-Neuve, Belgium.

No GMP inspections are deemed necessary.

### *Overall conclusions on administrative particulars*

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

# Part 2 – Quality

## *Composition*

Suvaxyn Circo+MH RTU is a bivalent vaccine containing inactivated *M. hyopneumoniae* strain P-5722-3 and inactivated chimeric cPCV1-2 as active ingredients providing active immunisation against two important porcine pathogens. The pharmaceutical form is an emulsion for injection which contains thiomersal as preservative and an adjuvant system based on squalane, incorporated in an oil-in-water emulsion (SP Oil), containing also poloxamer 401 and polysorbate 80.

Each 2 ml dose of vaccine contains from 1.5 to 3.8 relative potency (RP) of inactivated *M. hyopneumoniae* (M. hyo) strain P-5722-3 (determined by ELISA antigen quantification compared to a reference vaccine), and from 2.3 to 6.4 RP of inactivated cPCV1-2.

## *Container*

Suvaxyn Circo+MH RTU vaccine is presented in 25-dose (50 ml), 50-dose (100 ml) and 125-dose (250 ml) presentations. The vaccine is filled into HDPE vials closed with a chlorobutyl rubber stopper and sealed with an aluminium cap. The HDPE vials comply with the requirements of European Pharmacopoeia (Ph. Eur.) monograph 3.1.5. and the rubber stoppers comply with the requirements of Ph. Eur. monograph 3.2.9.

## *Development pharmaceutics*

The cPCV1-2 antigen included in Suvaxyn Circo+MH RTU vaccine is a known active substance. The cPCV1-2 was constructed by cloning the open reading frame 2 (ORF2), encoding the immunogenic capsid gene of the pathogenic PCV2 into the genome backbone of the non-pathogenic PCV1. In this chimeric construct, the PCV2 capsid gene does confer neither virulent nor infectious phenotype to the recombinant virus. The PCV1 is non-pathogenic as well. The cPCV1-2 antigen is produced on a porcine kidney (PK-15) cell line.

The M. hyo antigen is produced in medium supplemented with commercial porcine serum, which may contain large amounts of anti-PCV2 antibodies. As a result of the M. hyo antigen production process, these anti-PCV2 antibodies may be present in the final M. hyo antigen fluids and may as such interfere with the PCV2 potency assay performed on the finished product. As it is not possible to source a sufficient quantity of PCV2 antibody negative serum (due to the ubiquitous nature of PCV2), Zoetis has developed a method to remove porcine IgG (including anti-PCV2 antibodies) from the M. hyo antigen fluids. This method ("protein A treatment") consists of passing the inactivated, clarified M. hyo antigen fluids through a chromatography column packed with protein A resin (medium for capturing immunoglobulins (IgG)). An in-process test has been implemented to quantify IgG in the post-protein A treated M. hyo antigen fluids in order to ensure process performance.

M. hyo is inactivated with binary ethylenimine (BEI), produced *in situ* by treatment with bromoethylamine hydrobromide (BEA) 1M solution. cPCV1-2 is inactivated using a known inactivation procedure using betapropiolactone (BPL) as inactivating agent.

The proposed adjuvant system is based on squalane, incorporated in SP Oil, containing also poloxamer 401 and polysorbate 80.

Thiomersal is added as preservative.

## *Method of manufacture*

**M. hyo antigen**

Master seed (MS) or working seed (WS) is inoculated into pleuro-pneumoniae-like organisms (PPLO) complete medium and scaled up in culture vessels prior to culture in the production fermentor. At the end of the growth period in the production fermentor, the pH of the culture is adjusted and the culture inactivated with BEI (in situ prepared from BEA). Following inactivation, the antigen fluids are neutralised with sodium thiosulfate and subsequently clarified by depth filtration followed by 0.2 µm filtration. The clarified antigen fluids are submitted to protein A chromatography. The protein A treated M. hyo fluids are sterilised by filtration through a 0.2 µm filter.

Bioburden can be introduced into the M. hyo manufacturing process during the protein A chromatography step. Therefore, a bioburden test has been implemented prior to the sterile filtration step, with the upper bioburden limit set at $2 \times 10^6$ cfu/ml. However, considering the potentially high bioburden level prior to the sterile filtration step (i.e. up to $2 \times 10^6$ cfu/ml) and given the fact that this filtration step is the only sterilisation step in the manufacturing process of the M. hyo antigen, as an additional precautionary measure, the CVMP has recommended that a pre-filtration step is introduced prior to the final sterile filtration step in the M. hyo antigen production process in line with Ph. Eur. chapter 5.1.1. This additional step has been set as a condition to the marketing authorisation that will be implemented through an appropriate variation by the applicant in due course.

The maximum number of M. hyo subcultures from the MS for antigen production is MS+12.

The M. hyo inactivation process has been appropriately validated.

**cPCV1-2 antigen**

Scaled-up PK-15 cells are inoculated with cPCV1-2 seed and planted on microcarriers for growth. During the growth phase, up to four harvests of virus may be collected by adding fresh growth medium after each harvest. The obtained 1-fold antigen harvest stocks are concentrated (and purified) between 10-fold to 30-fold by filtration using 500 kDa membranes followed by diafiltration. The concentrated antigen fluids are inactivated with BPL in two stages. Inactivation is terminated by the addition of sodium thiosulfate.

The maximum passage level for antigen production is MS virus (MSV) +7.

The cPCV1-2 inactivation process has been appropriately validated.

**Finished product**

After blending of all constituents, the pH of the formulated blend is adjusted and the final blend is filled into HDPE vials.

## *Control of starting materials*

## *Active substance*

The preparation and testing of the master and working seed virus (cPCV1-2), the master and working seed bacteria (M. hyo), and the master and working cells (PK-15) is briefly described in the dossier and it is indicated that these seed lots have been controlled according to Ph. Eur. and current EU regulations.

## *Excipients*

The adjuvant system, SP Oil and the buffer system, phosphate buffered saline (PBS) used in Suvaxyn Circo+MH RTU vaccine comply with appropriate in-house specifications. The preservative thiomersal complies with the relevant Ph. Eur. monograph.

Information has been provided on the starting materials of both biological and non-biological origin used in the production of Suvaxyn Circo+MH RTU.

The starting materials of biological origin used in the production of Suvaxyn Circo+MH RTU vaccine are: squalane, foetal bovine serum (FBS), polysorbate 80, lactalbumin hydrolysate (LAH), protein A resin, microcarriers, OptiMEM x salts II solution, PPLO broth, porcine serum, porcine trypsin powder and yeast extract powder.

The starting materials of non-biological origin used in the production of Suvaxyn Circo+MH RTU vaccine are: cysteine hydrochloride monohydrate, glucose (anhydrous), glycerol, hydrochloric acid (concentrated), purified water, sodium chloride, sodium hydroxide, sodium thiosulfate, gentamicin sulphate, phenol red sodium salt, potassium chloride, potassium phosphate monobasic anhydrous, sodium hydrogen carbonate, sodium phosphate dibasic anhydrous, sodium phosphate dibasic heptahydrate, sodium dihydrogen phosphate monohydrate, sodium thiosulfate pentahydrate, poloxamer 401, water for injections and potassium phosphate monobasic anhydrous.

For the starting materials not listed in Ph. Eur., in-house specifications and certificates of analysis detailing controls tests have been provided.

Information has also been provided on the in-house prepared media and solutions used in the production of Suvaxyn Circo+MH RTU vaccine, i.e. antifoam solution, cysteine chloride solution, anhydrous dextrose

solution, PPLO broth porcine, yeast extract solution, sodium hydroxide solution, hydrochloric acid solution, equilibration buffer, sodium thiosulfate 25% solution, BEA 1M solution, OptiMEM with 0.25% LAH, OptiMEM with 0.25% LAH/FBS/gentamicin, Eagle's medium with 0.05% LAH, 0.01M Phosphate Buffer Saline (PBS), gentamicin sulphate solution, 1M sodium thiosulfate solution, 10X trypsin solution with EDTA, SP oil solution and thiomersal 1-10% solution.

The qualitative and quantitative composition of these media and solutions, the methods of preparation (including sterilisation procedure), controls and tests performed and the storage conditions have been described in the dossier.

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

The starting materials of animal origin comply with the note for guidance on minimising the risk of transmitting animal spongiform encephalopathies agents via human and veterinary medicinal products (EMEA/410/01-rev.3).

The overall transmissible spongiform encephalopathy (TSE) risk associated with this vaccine is considered negligible.

## *Control tests during production*

Tests performed during production of the M. hyo antigen are: gram stain, purity and colour changing units (CCU) titration on fermentation fluids before inactivation, completeness of inactivation immediately after inactivation, residual sodium thiosulfate after neutralisation and sterility, antigen content and swine IgG content after final sterilisation.

Tests performed during production of the cPCV1-2 antigen are: sterility, titration and identity on concentrated antigen fluids prior to inactivation; and sterility, completeness of inactivation, residual sodium thiosulfate, antigen content and protein content on neutralised, inactivated antigen fluids.

Test methods have been described (and validated) and specifications have been presented. Batch results are presented for two batches each of both M. hyo and cPCV1-2 antigen, all showing compliance with the specifications.

## *Control tests on the finished product*

The following tests are performed on the finished product: description, sterility, M. hyo identity/potency, cPCV1-2 identity/potency, pH, thiomersal content, squalane quantification and dynamic viscosity. A description of the methods (including validation data) and the specifications are provided.

Batch release data are presented for nine consecutive batches of Suvaxyn Circo+MH RTU vaccine, all showing compliance with the proposed specifications.

## *Stability*

Stability data have been presented for both the M. hyo and cPCV1-2 antigens, supporting a shelf-life of 24 and 27 months respectively.

Stability data have also been presented for nine consecutive (production scale) batches of finished product. The data cover 21 months of storage between +2 °C to +8 °C. Based on these data an 18 months shelf-life can be granted.

### *Overall conclusions on quality*

The composition and characteristics of the vaccine were sufficiently described. Detailed information has been provided on the starting materials. The manufacturing process was described and validated.

In-process controls tests and release tests on final product are properly described and are considered sufficient to control the quality of the vaccine.

The main risks concerning TSE are considered negligible.

The proposed shelf-life of 18 months at +2 °C to +8 °C for the finished product is considered acceptable based on the data available.

CVMP concluded that the presented documentation is considered sufficient to ensure the quality of the finished product, however, considering the potentially high bioburden level prior to the M. hyo sterile filtration step, a pre-filtration step should be performed in the M. hyo antigen production process in accordance with Ph. Eur. 5.1.1. The updated documentation to describe and validate the pre-filtration step introduced before the filtration step should be provided by means of an appropriate variation. Furthermore, the applicant should demonstrate that the pre-filtration step does not adversely affect the quality and the quantity of the active ingredient in the final product. This step has been set as a condition to the marketing authorisation and is mentioned in annex II of the product information.

## Part 3 – Safety

Two (2) laboratory and four (4) field safety studies were performed in accordance with Ph. Eur. monograph 0062 on Vaccines for veterinary use and Ph. Eur. monograph 5.2.6 on Evaluation of safety of veterinary vaccines and immunosera. In addition one field study was conducted in the US. Moreover, the laboratory trials have been conducted in accordance with Good Laboratory Practice (GLP) requirements and field studies were compliant with Good Clinical Practice (GCP).

Except where otherwise indicated, vaccinations were performed in accordance with the proposed vaccination scheme and administration route: IM single vaccination of 2 ml from 3 weeks of age. The health status of the animals included in the studies was tested individually and no data on the herd were provided.

### *Laboratory tests*

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

The safety of the administration of one dose and of an overdose of vaccine was evaluated in a total of 36, 17–22-day-old crossbred piglets (the most sensitive category of target species) which were seronegative for PCV2 and M. hyo before vaccination. The animals sourced from a holding where no recent clinical history of infection by *Haemophilus parasuis*, porcine reproductive and respiratory syndrome virus (PRRSV), M. hyo and porcine circovirus (PCV) was recorded. Two (2) groups of 12 pigs each were treated IM respectively with 1 dose of Suvaxyn Circo+MH RTU at the maximum potency (PCV of 6.42 RP and M. hyo of 3.84 RP) and a double dose of the same batch of Suvaxyn Circo+MH RTU; 1 group of 12 pigs was kept as unvaccinated control and was administered with saline solution.

Animals were monitored over 28 days. No clinical signs and no impact on growth were reported. However, transient increase in body temperature was very common during the first 28 hours after vaccination. Two (2) piglets, one from each group reported local reaction at the site of injection in form of swelling areas not exceeding 2 cm of diameter and disappearing within 2 days. At the end of the

observation period microscopic analysis of the injection site, showed in all pigs minimal to moderate lymphocytic and macrophage infiltration, in some cases associated to multi nucleated giant cells and minimal fibroplasia. Information on the safety profile of the vaccine were collected also from one efficacy laboratory study where 3 pigs reported mild hypersensitivity-like reactions such as vomiting or depression after vaccination. Since the vaccine was administered at weaning those signs might be due to physiological induction of cortisol secretion and intestinal modification that frequently cause diarrhoea in piglets, however a differential diagnosis with anaphylactic reactions needs to be taken into consideration and a proper warning has been included in the SPC.

In conclusion, result showed that the administration of a single dose of Suvaxyn Circo+MH RTU is considered safe. Adverse reaction such as limited local reactions, transient increase in temperature and potential anaphylactic reaction are adequately addressed in the SPC.

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

A study was provided aiming at investigating the safety of the repeated administration of one dose of Suvaxyn Circo+MH RTU in 16–20-day-old pigs. A total of 24 piglets were enrolled in the study. A group of 12 pigs was administered the vaccine IM 2 times, 14 days apart. A group of 12 pigs was left unvaccinated as control group and saline solution was administered. The farm from where the piglets came and piglets' characteristics, as well as the vaccine batch were similar to the previous study. Rectal temperature was significantly higher after the first injection but not after the second. No other general reactions were reported. There was no difference in weight gain between vaccinated and control pigs during a one-month observation period. Local reactions were observed in the 17% (2 out of 12) of the vaccinated animals, in form of swelling areas not exceeding 2 cm of diameter, 4 days after the second injection. The swellings disappeared in 6 days.

In conclusion, the safety of repeated administration of one dose of the vaccine has been shown. A transient increase in temperature and limited local reactions were observed and are adequately addressed in the SPC.

## *Examination of reproductive performance*

No study has been carried out to assess the safety in pregnant or lactating sows, or in breeding boars. Therefore Suvaxyn Circo+MH RTU cannot be recommended for use in these categories of the target species and this has been addressed in the SPC.

## *Examination of immunological functions*

No specific tests on immunological functions were carried out and this is acceptable because the inactivated antigens contained in the vaccine, as well as the adjuvant system used are not known to have an impact on immune function.

## *Special requirements for live vaccines*

Not applicable.

## *Study of residues*

Not required.

The active substances of biological origin intended to produce active immunity do not fall within the scope of Regulation (EC) No. 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin.

The excipients, including adjuvants, are either allowed substances for which table 1 of the annex to Commission Regulation (EU) No. 37/2010 indicates that no maximum residue limits (MRLs) are required, or are considered as not falling within the scope of Regulation (EC) No. 470/2009 when used as in this product.

The gentamicin used in cell culture is present at low residual levels in the finish medicinal product. The expected levels of gentamicin at injection site are more than 100 times below the porcine muscle MRL.

The withdrawal period is set at zero days.

## *Interactions*

No data concerning interactions with other veterinary medicines were provided and the respective standard warnings are stated in the SPC.

## *Field studies*

Five (5) field studies were performed with the aim to evaluate the safety of Suvaxyn Circo+MH RTU in 3-week-old commercial piglets. Four (4) GCP-compliant studies were conducted in Europe and one (1) study was a multisite study conducted in US (3 different sites) and data from this last study can only be considered as supportive information.

A field trial was conducted in a commercial farm in the Netherlands to evaluate both safety and efficacy of Suvaxyn Circo+MH RTU. A total of 130 piglets was enrolled. A group of 65 animals was administered by IM injection a dose of vaccine at medium potency (PCV2 of 3.4 RP of and M. hyo of 2.4 RP) and a group of 65 animals was used as control group and treated with saline solution. General signs of safety were monitored by close surveillance during the first hour after vaccination, temperature measurement within 4 hours and weight gain was recorded over a 1-month period after vaccination. Local reactions were monitored until disappearance. Besides, the percentage of animals receiving individual antibiotic treatment, health events and mortality over the 30-day period was monitored as well. No signs of anaphylactic reaction were observed after vaccination. The average rectal temperature was significantly increased 4 hours post vaccination (average increase 0.6 °C) in the vaccine group when compared to the control group, and returning to normal the day after. Swelling at the injection site was observed in 13% (2 out of 13) of the vaccinated animals one day post vaccination, with a diameter not exceeding 0.5 cm and disappearing within two days. No redness, pain or heat at the injection site was observed at any time point. Body weights between treatment groups did not differ significantly during the trial. Any other health event observed during the study was not related to the product.

In conclusion, it has been demonstrated that a single dose of Suvaxyn Circo+MH RTU was safe when administered by IM injection to approximately 3-week-old piglets under field conditions.

A second field trial was conducted in a commercial farm in the Netherlands to evaluate both safety and efficacy of Suvaxyn Circo+MH RTU. A total of 399 21-day-old piglets were enrolled in this study. A group of 200 animals was administered by IM a dose of vaccine at below minimum release potency (PCV2 of 1.46 RP and M. hyo of 1.16 RP). This is considered acceptable because the design of this study was done to assess also the efficacy of the product. A group of 199 animals was used as control group and treated with saline. General signs of health were monitored around the time of vaccination, and weight gain was monitored the day before vaccination, at the transfer to the fattening stable and at slaughter. No

anaphylaxis-like symptoms were observed after vaccination in the vaccine. Body weights between treatment groups did not differ significantly.

In conclusion, it has been demonstrated that a single dose of Suvaxyn Circo+MH RTU was safe when administered by IM injection to approximately 3-week-old piglets under field conditions.

A third field trial was conducted in a commercial farm in the Netherlands to evaluate both safety and efficacy of Suvaxyn Circo+MH RTU. A total of 130 piglets were enrolled in this study. A group of 65 animals was administered a dose of vaccine at medium potency (PCV2 of 3.4 RP and M. hyo of 2.4 RP) and a group of 65 animals was used as control group and treated with saline solution. General signs of safety were monitored by close surveillance the first hour after vaccination, temperature measurement from the day before until 4 days after vaccination and by weight gain over a 1 month period after vaccination. Local reactions were monitored until disappearance. Besides antibiotic treatment, health events and mortality over the 30-day period were monitored as well. No anaphylaxis-like symptoms were observed after vaccination, in neither the vaccine nor the control group. The average rectal temperature was significantly increased 4 hours post vaccination (average increase 1.0 °C) in the vaccine group when compared to the control group. That difference was no longer present one day post vaccination. No swelling, redness, pain or heat at the injection site was observed at any time point. Body weights between treatment groups did not differ significantly just prior to vaccination and 14 days thereafter. Any other health event observed during the study was not related to the product.

In conclusion, it has been demonstrated that a single dose of the Suvaxyn Circo+MH RTU was safe when administered under field conditions.

The fourth field trial was conducted in a commercial farm in the United Kingdom to evaluate the safety of Suvaxyn Circo+MH RTU. A total of 130 piglets were enrolled in this study. A group of 65 animals was administered a dose of vaccine at medium potency (PCV2 of 5.92 RP and M. hyo of 2.92 RP) and a group of 65 animals was used as control group and treated with saline solution. General safety signs were monitored by close surveillance during the first hour after vaccination. The temperature was measured from the day before vaccination until 4 days post vaccination and weight gain was monitored for 1 month post vaccination. Local reactions were observed until disappearance. Besides antibiotic treatment, health events and mortality for 30 days were monitored. No anaphylaxis-like symptoms were observed after vaccination, in neither the vaccine nor the control group. The average rectal temperature was not significantly different at any time point measured. Four (4) hours post vaccination the average increase was 0.4 °C in the vaccinated group when compared to the control group. Swelling at the injection site was observed in 20% (3 out of 15) of the animals in the vaccinated group one day post vaccination, with a diameter not exceeding 0.5 cm. Two (2) days after vaccination, these swellings were no longer detected. No redness, pain or heat at the injection site attributable to the candidate vaccine was observed at any time point. Body weights between treatment groups did not differ significantly at day 1 and after one month post administration. Any other health event observed during the study was not related to the product. No mortality was observed in the study.

In conclusion, it has been demonstrated that a single dose of the Suvaxyn Circo+MH RTU was safe when administered by IM to 3-week-old piglets under field conditions.

A fifth field study provided was conducted 3 farms located in 3 different states in the US, with the aim of evaluating the safety of Suvaxyn Circo+MH RTU. Three (3) groups of piglets were reared in each farm. In total, 474 pigs were enrolled in the study. Three hundred sixteen (316) pigs were vaccinated with one of 2 batches below the maximum recommended potency (PCV2 RP of 3.4 and M. hyo RP of 2.4 or PCV2 RP of 3.9 and M. hyo RP of 2.2). A hundred fifty eight (158) pigs were left unvaccinated as controls and were administered with saline solution. In 2 farms weaned piglets were vaccinated respectively at 16–25 days

of age and in the third farm 17–21-day-old animals were vaccinated one day before weaning. Clinical signs were recorded for all animals 1 hour and 6 hours post vaccination and on days 1, 7, 13 or 14 and 21 post vaccination. Injection site observations were performed in all animals 1 and 7 days post vaccination. Animals which developed local reactions after vaccination were monitored daily until resolution of the reaction.

Piglets were exposed to natural infectious agent (PRRS or bacteria). The number of vaccinated piglets reporting diarrhoea or respiratory impairment was not statistically different between vaccinated and controls. Mild and transitory local reactions were reported in 1% of piglets (4 out of 316).

In conclusion, the safety evaluation of the vaccine was favourable. Nevertheless, the study was conducted in US using a vaccine similar to the proposed product and therefore, results from this field study can only be considered as supportive.

## *User safety*

A user risk assessment compliant with the CVMP Guideline for user safety for immunological veterinary products (EMEA/CVMP/IWP/54533/2006) was provided. Potential risks were identified.

The active substances of Suvaxyn Circo+MH RTU are inactivated and are not cause of concern to the user.

The excipients used in the vaccine are either authorised food additives in the EU or other substances included in table 1 (allowed substances) of annex to Commission Regulation (EU) No. 470/2009 with a no MRL-required status, or included in the list of substances not falling within the scope of Regulation (EC) No. 470/2009.

The adjuvant is composed of squalane, polysorbate 80 and poloxamer 401. The last two mentioned substances are known to trigger anaphylactic type reactions in humans. However, it is reasonable to conclude that the risk of anaphylactic type reactions after parenteral exposure to the user is minimal.

Risks are essentially associated with handling and accidental self-injection. Exposure to vaccine is limited to the 2 ml volume of a vaccine dose.

The vaccine does not contain any mineral oil material. In safety studies in piglets, the vaccine was well tolerated at the injection site and the product is sterile which decreases the risk of infection upon self-injection.

Consequently, the risk is considered to be minimal and no specific warning has been included in the SPC.

## *Environmental risk assessment*

A risk assessment has been provided in compliance with the CVMP Guideline on the environmental risk assessment of immunological veterinary medicinal products (EMEA/CVMP/074/95).

*Hazard identification:*

The two vaccine strains included in the product are inactivated. The adjuvant is constituted by a mixture of squalane and non-ionic surfactants which are included in the list of compounds for which no MRL is required. Thiomersal ($C_9H_9HgNaO_2S$), mercury containing organic compound is the most toxic for the environment.

*Exposure to hazard:*

Since the product is used in piglets and administered by IM, direct exposure of the environment does not

occur. Any unused product or waste should nevertheless be disposed of by the appropriate channels. The only toxic component that would be excreted by vaccinated pigs is the mercury from the thiomersal. A maximum of 12.5 mg of mercury could be released in the environment if a bottle is spilled which would be dispersed by water and will not reach significant concentrations in the environment.

Based on the data provided the ERA can stop at phase I. Suvaxyn Circo+MH RTU is expected to pose a negligible risk for the environment when used according to the SPC.

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

Not applicable.

## *Overall conclusions on the safety*

The safety of Suvaxyn Circo+MH RTU in target species was investigated in 2 GLP-compliant laboratory safety studies, performed in accordance with Ph. Eur. monograph 0062 on Vaccines for veterinary use and Ph. Eur. monograph 5.2.6 on Evaluation of safety of veterinary vaccines and immunosera.

Three (3) GCP-compliant field safety and efficacy studies and 1 GCP-compliant field safety study were also provided. One (1) additional field study was provided however as it was conducted in US, results can only be considered as supportive.

The safety of a single dose administration and of a 2-fold overdose administration of Suvaxyn Circo+MH RTU was demonstrated. In addition, the safety of repeated administration of one dose of the vaccine has been shown.

Transient hyperthermia up to 1 °C after vaccination was very commonly reported. Increase in temperature of 2 °C was also commonly reported. Hyperthermia shown in the field trials was higher than in laboratory studies.

Immediate mild hypersensitivity-like reactions may occur uncommonly after vaccination and anaphylaxis may occur in very rare cases. In case of such reactions, appropriate treatment is recommended.

Local tissue reactions in the form of swelling below 2 cm in diameter were observed commonly at the injection site, due to mild inflammatory response. The local tissue reactions disappeared within 2 days. The adverse reactions are appropriately reflected in the SPC.

Potential effect on reproductive performance were not investigated on the basis that the vaccine is not intended for breeding animals and this is considered acceptable.

Residue studies are not required. The withdrawal period is set at zero days.

The user safety has been adequately addressed. The CVMP concluded that the user safety for this product is acceptable when used as recommended in the SPC.

Suvaxyn Circo+MH RTU is expected to pose a negligible risk to the environment when used according to the SPC.

The CVMP concluded that the safety of Suvaxyn Circo+MH RTU has been demonstrated when used as recommended in the SPC.

# Part 4 – Efficacy

## *PCV2*

## *Introduction and general requirements*

PCV2 is a virus involved in a large array of syndromes which has been recently classified (Segalés, 2012) in the 6 main following groups: PCV2 subclinical infection (PCV2-SI), PCV2 systemic disease (PCV2-SD), PCV2 lung disease (PCV2-LD), PCV2 enteric disease (PCV2-ED), PCV2 reproductive disease (PCV2-RD), porcine dermatitis and nephropathy syndrome (PDNS).

A correlation has been demonstrated between the amount of PCV2 virus and the severity of disease (Grau-Roma et al., 2009).

The diagnosis of the diseases relies on the presence of clinical signs associated with the PCV2 load and typical histopathologic changes of the target tissues (Sorden's criteria).

Vaccination is expected to reduce viral load in blood and lymphoid tissues and virus faecal shedding caused by infection with PCV2.

The efficacy of Suvaxyn Circo+MH RTU was investigated in five (5) laboratory studies compliant to the Ph. Eur. monograph 0062 on Vaccines for veterinary use and Ph. Eur. monograph 5.2.7 on Evaluation of efficacy of veterinary vaccines, four (4) other studies to evaluate the efficacy and in three (3) GCP compliant field studies.

In laboratory studies, piglets were challenged by a PCV2a strain with the aim to assess the onset of immunity (OOI) and by a PCV2b strain with the aim to assess the duration of immunity (DOI) as well as the interference with maternally derived antibodies (MDA). Since cross protection is described in literature against PCV2b and PCV2a, the use of those different challenge strains is acceptable. The route of administration of the challenge was changed over the studies and this is acceptable. As further described below, challenges were carried out 1 time by intranasal route (IN) or 4 times by IM and IN route.

In the laboratory studies, clinical parameters were monitored by validated tests (RT-PCR, histology lesions, serology and immunochemistry). In field studies zootechnical parameters such as antibiotic consumption, mortality, and weight gain were also monitored.

## *Laboratory trials*

**Onset of immunity**

Two (2) laboratory studies were performed with the aim to establish the OOI of the PCV2 part of the vaccine. Animals enrolled in these studies were tested to be PCV2 antibody free and PCV2 DNA free in the serum before vaccination.

In the first study provided a total of 120 3-week-old pigs were enrolled. Four (4) groups of 24 pigs each were treated with vaccines containing different amounts of PCV2 antigens below the minimum potency proposed (RP from 0.22 to 2) by IM, one group of 24 pigs was used as control group and vaccinated with a product containing only the M. hyo antigen at high potency (RP of 1.71) by IM. Piglets were challenged at 6 weeks with PCV2a virulent strain ($10^5$ $FAID_{50}$ (fluorescent antibody infectious dose/ml) by 2 different administration routes: 1 ml IM and 2 ml IN. Animals were observed for clinical signs for 3 weeks after challenge and sacrificed at day 45. After vaccination a total of 4 pigs (distributed in 3 groups of 24 vaccinated animals) expressed signs of anaphylactic type reactions such as vomiting, diarrhoea or

depression and erythema. The challenge also caused frequent mild anaphylactic-type reactions. One week after challenge, the viraemia was lower in the vaccinated group than in the control group (by a mean of $10^4$ copies genome of PCV2a) and was always null at least in 60% of animals. The virus faecal shedding was decreased in the same percentage of animals during the 2nd and 3rd week after challenge (around 1 DNA copy instead of 20,000 DNA copies in control animals).

During the study, 5 piglets were found viraemic before day 21, confirming that PCV2 natural infection circulated before challenge, however the study is acceptable since the validity criteria set by the applicant were met as only less than 10% of pigs were PCV2 positive (by real-time PCR) and the mean PCV2 antibody titer in the control group remained negative until challenge.

The second study provided presented with a similar study design to the previous study (24 pigs allocated in 4 groups vaccinated with different amount of PCV2 antigen and in 1 control group), the same batches of vaccines and a similar challenge (final titer of $10^5$ $FAID_{50}$/ml). Thirty nine (39) out of 96 vaccinated animals presented signs of anaphylactic reaction after challenge. This was a result of the higher protein content challenge being delivered IN/IM to pigs that were uncharacteristically reactive. Results showed that 1 week after challenge viraemia was decreased by about $10^3$ genome copies in 50% of the vaccinated pigs and the other 50% did not show any viraemia. Virus shedding was reduced in each vaccinated group either in the quantity of the virus excreted (by about $10^3$ genome copies) and in the percentage of pigs shedding the virus (25% to 43%) compared to the percentage of control pigs (83% were shedding the virus). Moreover, the percentage of animals with respectively lymphocytic depletion and histiocytic replacement in the lymphoid organs were decreased in all vaccinated groups from 48% to 4–12% and from 39% to 4–8% compared to the control group. In the group of pigs treated with the vaccine at the highest potency no histiocytic lesions were detected. The virus load in tonsils was also significantly decreased in all the vaccinated groups.

A PCV2 natural infection was detected in one vaccinated pig 1 week before challenge, however the study was considered acceptable due to absence of seroconversion in the control group from the same pen.

In conclusion, in the first laboratory study presented the OOI of the PCV2 part of the vaccine was demonstrated in 6-week-old piglets, 3 weeks after vaccination with respect to reduction of PCV2 viremia and PCV2 virus shedding. In the second laboratory study presented the OOI was confirmed 3 weeks after vaccination in pigs of the same age, regarding reduction of PCV2 viraemia, PCV2 shedding and also reduction of lesions and PCV2 load in lymphoid tissues.

**Duration of immunity**

One (1) laboratory study was provided with the aim to establish the DOI at 23 weeks post vaccination. A total of 60 crossbred piglets were enrolled in this study. Thirty (30) crossbred piglets were treated at 3 weeks of age with 2 ml of vaccine below the minimum of potency (RP of 1.03) by IM route and 30 pigs were used as controls (treated with a placebo). Twenty three (23) weeks later, animals were challenged by IN administration with 4 ml of a PCV2b strain containing a titer of $10^{5.7}$ $TCID_{50}$/ml. No signs of anaphylactic reactions were recorded post vaccination. In the vaccinated group, viraemia was prevented in 100% of the pigs and the number of pigs shedding the virus was significantly reduced in the vaccinated pigs (20.7%) compared to the control group (60.7%). The number of pigs presenting lymphocyte depletion and histiocytic replacement in the lymphoid organs was similar (about 10%) in both vaccinated pigs and controls, however detection of PCV2b load in the lymphoid organs was lower in vaccinated pigs and this difference was statistically significant. Results showed that vaccinated and control pigs had a similar growth rate.

In conclusion, the DOI for the PCV2 part of the vaccine was demonstrated at 23 weeks post vaccination, on the basis of reduction of PCV2 viraemia, PCV2 shedding and viral load in lymphoid organs.

**Interference with maternally derived antibodies**

Two (2) studies were provided with the aim to evaluate the potential interference of MDA against the efficacy of the PCV2 part of the vaccine.

In the first study provided, sixty (60) 3-week-old MDA-positive (MDA+) piglets, (1.1-1.2 S/P ELISA) were enrolled. Thirty (30) piglets were treated by IM route of administration with a vaccine containing the PCV2 antigen below the minimum of potency (RP of 0.97) and M. hyo at high potency (RP of 2.03), and 30 piglets were used as controls and treated by IM only with a product containing only M. hyo antigen at high potency.

The animals were challenged 5 weeks after vaccination with a PCV2b virulent strain which was administered by IN (2 ml) and by IM (1 ml). Three (3) piglets out of 60 showed signs of anaphylactic reaction owing to the protein amount of the challenge material. After challenge, PCV2 antibodies increased significantly in the vaccinated group compared to the control group. Vaccination did not significantly decrease the viraemia and faecal shedding when piglets had MDA. This study was considered invalid by the applicant due to the presence of MDA level during the challenge that interfered with the PCV2 viraemia (only 31% of the controls were PCV2 viraemic). However, results after the challenge (% of viraemic and % of pigs shedding the virus) in control pigs were similar to those presented in the following study.

Due to the high antibody levels in all groups of pigs, the protective potential of the vaccine might have been masked and a conclusion could not be drawn.

In the second study provided, the interference of MDA was investigated in 23–27-day-old pigs with a lower level of MDA present at the challenge timepoint (0.15 S/P ELISA) than in the first study. The level of MDA was also lower at the time of vaccination (0.74 S/P ELISA). A total of 96 pigs were enrolled in this study. Two (2) groups of 24 pigs each were treated by IM with different vaccines containing below minimum potency (PCV2 RP of 1.01) and minimum potency (PCV2 RP of 2.33) of the final formulation. One (1) group of 24 MDA-negative (MDA-) pigs was treated with the vaccine at lower strength (PCV2 RP of 1.01) but due to the study design and study conditions, statistical comparison with other groups was not relevant because it did not form part of the same randomization process and results from this group could not be taken into account. One (1) group of MDA+ pigs was used as a control group and treated with a placebo by IM.

All 4 groups, were challenged 35 days after vaccination by administration of PCV2b strain (about $10^6$ $TCID_{50}$) by both IN and IM routes. The challenge was not strong enough to cause pathological lesions but 52% of the MDA+ control group showed viraemia. No MDA- unvaccinated control group was included in the study to compare the MDA interference on viraemia and virus shedding after challenge. The vaccine with low potency (PCV2 RP of 1.01) was not efficient enough to decrease viraemia, virus shedding and virus load in tracheobronchial, mesenteric and inguinal lymph nodes as well as in tonsil of MDA- animals. However, MDA+ pigs with a level of MDA at vaccination about 0.7 S/P ELISA, when treated with the vaccine at minimum release potency (PCV2 RP 2.33) showed a decrease in viraemia, viral load in lymphoid organs and in faeces when compared to MDA+ control group.

In conclusion, a statistically significant decrease in viraemia, viral load in lymphoid organs and faecal shedding was demonstrated after the challenge, in animals vaccinated with PCV2 vaccine at minimum release potency (RP of 2.33) in presence of MDA levels representative of field conditions at vaccination. Therefore no specific warning in the SPC is necessary.

**Other studies to evaluate the efficacy**

Four (4) studies were reported as GCP-compliant studies with the aim to evaluate the immunogenicity of

Suvaxyn Circo+MH RTU against viraemia, and lymphoid tissues and faecal shedding caused by PCV infection. In two (2) of these studies MDA+ piglets were challenged with both M. hyo and PCV2. Efficacy parameters were reduction of viral load in blood, of faecal shedding, of viral load in lymphoid tissues and average daily weight gain.

In the first study provided, 126 conventional piglets were enrolled. One hundred and twenty (120) piglets were treated IM with a commercial batch of the vaccine (PCV2 RP between 3.2 to 4.8) The pigs were challenged 42 days after vaccination at 3 weeks of age. Six (6) piglets were left untreated and unchallenged as sentinels. Results showed significant reduction of viral load in blood, of faecal shedding, and of viral load in lymphoid tissues in all vaccinated groups.

In conclusion, results demonstrate the efficacy of a single dose of Suvaxyn Circo+MH RTU with regard to the reduction of viraemia, virus load in lymphoid organs and faecal shedding.

In the second study provided, 208 conventional piglets were enrolled. One hundred sixty (160) animals were treated IM at the age of 3 weeks with a commercial batch of the vaccine (PCV2 RP between 3.4 and 4.7). A group of 40 pigs were left unvaccinated as control. All piglets were challenged 23 weeks after vaccination. A group of 8 piglets were used as sentinels. Results showed significant reduction of viraemia, of faecal shedding, and of viral load in lymphoid tissues in all vaccinated groups.

It can be concluded that the efficacy of Suvaxyn Circo+MH RTU was demonstrated, with regard to the reduction of viraemia and viral load in lymphoid tissues and faecal shedding caused by infection with PCV2. Moreover, the DOI was demonstrated at 23 weeks post vaccination.

In the third study provided, 248 conventional piglets of which 5 groups of 48 animals each were treated IM at the age of 3 weeks, including one group with a commercial batch of vaccine used according to the proposed product information (PCV2 RP of 4.7); eight (8) piglets were kept as sentinels. Sixty five (65) days after vaccination, pigs were PCV2b challenged. One week earlier, the pigs were M. hyo challenged. Significant reduction of PCV2 viral load in blood, of faecal shedding, of viral load in lymphoid tissues and PCV2-associated weight loss were shown for the vaccinated group.

In conclusion, the efficacy of Suvaxyn Circo+MH RTU against PCV2b challenge was demonstrated with regard to viraemia, viral load in lymphoid organs and faecal shedding. In addition, the vaccine was demonstrated to reduce PCV2 associated weight loss.

In the fourth study provided, 408 conventional piglets were enrolled. Eighty (80) pigs were treated at the age of 3 weeks with a commercial batch of vaccine (PCV2 RP of 4). Pigs were PCV2 challenged 65 days after vaccination. The piglets were also challenged by M. hyo 51 days after vaccination. Significant reduction of viral load in blood and improvement in average daily weight gain were shown for the vaccinated group compared to unvaccinated controls.

In overall conclusion, the OOI was demonstrated at 3 weeks and the DOI at 23 weeks post vaccination with regard to the PCV2 component with regard to reducing viraemia, viral load in lymphoid tissues and faecal shedding. The claim of decreasing the weight loss caused by PCV2 was not consistently supported.

Notwithstanding the challenge with PCV2 in the field studies, the vaccine batches below the minimum of the potency were able to consistently lower PCV2 viraemia and faecal shedding as well as the virus load in lymphoid organs. However, the experimental model used was unable to trigger clinical disease or growth stunting and even lymphoid lesions in 6-month-old pigs. Therefore, the clinical relevance of zootechnical parameters in vaccinated pigs is difficult to extrapolate. While more virulent models could have been used such as a combination of PCV2 and other pathogens (e.g. PPV, PRRS, etc.). The model presented in the dossier has already been previously established in relation to another vaccine (Suvaxyn PCV) and is acceptable.

## *Field trials*

Three (3) GCP-compliant field studies were conducted in 3 commercial pig farms in the Netherlands with the aim to evaluate the efficacy of Suvaxyn Circo+MH RTU. Those studies have been described in section "Field trials with PCV2 and M. hyo" under *Mycoplasma hyopneumoniae*.

## *Mycoplasma hyopneumoniae*

## *Introduction and general requirements*

M. hyo is involved in porcine enzootic pneumonia (PEP), a highly contagious and chronic lung disease associated with deterioration of zootechnical parameters.

The diagnosis of the disease is based on pulmonary signs or lung lesions detected at the slaughterhouse (expressed as percentage of lung lesion referring to the percentage of lung parenchyma with lesion) and confirmed by direct detection or isolation of M. hyo.

Vaccination is expected to restore zootechnical parameters which are correlated to a reduction of lung lesions.

The efficacy of the M. hyo part of the vaccine was demonstrated in 8 GLP and Ph. Eur.-compliant laboratory studies and 3 GCP-compliant field studies where zootechnical parameters such as the number of pigs receiving antibiotic treatment and the mortality were considered in addition to the observation carried out during laboratory studies (percentage of lung lesions and the body weight). All the parameters (gross pathology and serology) were fully technically validated.

The vaccine strain was derived from a North-American isolate from Nebraska. On the basis of literature (Thacker and Minion, 2012) this strain is considered relevant to the European SBV epidemiological context. The challenge was carried out with a M. hyo positive porcine lung homogenate collected at Iowa State University.

## *Laboratory trials*

**Onset of immunity**

Four (4) studies were provided in order to demonstrate the OOI of the M. hyo part of Suvaxyn Circo+MH RTU.

The first laboratory study provided was conducted with the aim of evaluating the efficacy of the M. hyo part of the vaccine when administered IM in pigs of 3 weeks of age. The study was designed in compliance with Ph. Eur. 2448. A total of 150 crossbred pigs were enrolled in this study. Four (4) groups composed of 30 animals each were vaccinated with 4 different strengths of antigen (M. hyo RP of 1.47, 0.92, 0.50, 0.23) and were challenged 4 weeks later by intratracheal administration of 10 ml of a lung homogenate ($10^3$ M. hyo/ml). One (1) group of 30 pigs was used as control and treated with a placebo, a further 9 animals were left untreated as sentinels.

Animals were not exposed to M. hyo prior to challenge as demonstrated by negative tests (serology and M. hyo DNA) and absence of M. hyo lesions in sentinels. The control piglets, challenged by the intratracheal route with 10 ml of a M. hyo lung homogenate ($10^3$ CCU/ml) developed lung lesions (9.3%) and M. hyo antibody response.

Piglets vaccinated with vaccines with very low up to medium amounts of M. hyo antigens (RP from 0.23 to 0.92) had significantly lower percentage of lung lesions compared to controls. The load of M. hyo in the

nose was not reduced however the reduction of M. hyo load in the trachea was statistically significant (but narrowly decreased) in 3 out of 4 groups of vaccinated piglets ($7x10^6$ in control pigs versus $3–4x10^6$ M. hyo DNA copies/ml in vaccinated groups).

On the other hand, the percentage of lung lesions in piglets vaccinated with the highest potency vaccine was reduced but not significantly reduced compared to the control group and their M. hyo antibody level (by ELISA) observed post challenge was significantly higher compared to the other vaccinated groups.

Consequently, it can be concluded that no antigen dose response related to the protection against lung lesions could be established on the basis of the above study.

The second laboratory study provided was designed to investigate the interference of PCV2 antigen on pigs immunised by a vaccine containing M. hyo vaccine antigen below the minimum potency (0.8 RP). A total number of 160 pigs were enrolled in this study. Three (3) groups of 30 animals each were vaccinated by IM with different vaccines with a fixed quantity of M. hyo antigen and different quantities of PCV2 antigen at the minimum of potency or below (RP from 1 to 2.4). These 3 groups were compared to a group of 30 pigs vaccinated with a vaccine containing M. hyo antigen (RP of 0.8) without PCV2 antigen to investigate the PCV2 interference in the vaccine and to a group of 30 pigs vaccinated with PCV2 antigen only to investigate the protection afforded by M. hyo vaccination. The animals were challenged by the intratracheal administration of 10 ml of $10^3$ CCU M. hyo/ml on 2 consecutive days. The challenge (strain 11/232 Lot LI38 (5/11/05) was modified and intensified ($5.5 .10^3$ CCU/ml) in comparison with the first study and this is considered acceptable. Ten (10) pigs were left untreated as sentinels.

Animals were not exposed to M. hyo prior to challenge as demonstrated by negative tests (serology and M. hyo DNA) and absence of M. hyo lesions on sentinels. The challenge met applicant's validity criteria since observations of the control group showed a mean of 7% of lungs with gross lesions 4 weeks post challenge and cough from 12 days post challenge onwards. The percentage of lung lesion was equally significantly reduced in each of the vaccinated groups to 1.1%–2.3% against results in the control group. However, M. hyo DNA (by PCR) and M. hyo antigens (by IHC) were found at similar levels in the lungs of vaccinated and control piglets and the body weight gain was also similar between groups. After challenge a higher level of antibodies was found in vaccinated pigs than in controls, showing that the vaccine was stimulating an anamnestic antibody response.

In conclusion, the study demonstrated that a vaccine with a low dose of M. hyo antigen (RP 0.8, lower than minimum potency release) reduced the mean percentage of lung lesions but had no effect on the colonisation of lungs by M. hyo. Furthermore, comparison of doses of challenge used in this and the first study did not show a dose-response relation in the protection against lung lesions.

The third laboratory study was designed with the aim to confirm the OOI of the M. hyo part of the vaccine 3 weeks after vaccination. A total of 155 crossbred M. hyo seronegative piglets were enrolled in the study. Four (4) groups of 30 pigs each were vaccinated with 4 different M. hyo strengths (RP from 0.23 to 1.57) and were challenged 3 weeks later by intratracheal administration of 10 ml of a lung homogenate ($5x10^3$ M. hyo/ml). A fifth group of 30 pigs was treated with a placebo and used as control group. Six (6) animals were left untreated as sentinels. An outbreak of intercurrent respiratory infections by *S. suis* and *P. multocida* began 5 days after challenge and increased the percentage of the lung lesions up to 45% in controls.

Vaccinated pigs showed protection by a decrease of 50% the percentage of lung gross lesions compared to controls which was statistically significant, however such result was not dose-response related. The M. hyo load of nasal cavities increased statistically significantly only 29 days after challenge in control piglets and this increase did not occur in vaccinated animals. There was no difference of M. hyo load in the lungs between the vaccinated and control groups 50 days after vaccination (i.e. 30 days after challenge).

Piglets seroconverted 3 weeks after vaccination (00.3 < S/P < 00.4) prior to challenge and at necropsy (4 weeks post challenge). All vaccinated groups had significantly higher M. hyo antibody titers compared to the controls (S/P of 0.5 versus 2).

In conclusion, the OOI was established at 3 weeks after vaccination and no dose-response relation was observed.

The fourth laboratory study provided aimed to demonstrate the OOI of the M. hyo part of the vaccine in 3-week-old pigs at 3 weeks after vaccination.

Seventy (70) crossbred piglets were enrolled. Two (2) groups of 35 animals each were vaccinated with a low potency vaccine (M. hyo RP of 0.84) and were challenged 3 weeks later on 2 consecutive days. On the day of the challenge 10 ml of a lung homogenate (titer of $5x10^2$ CCU/ml M. hyo/ml) was administered intratracheally. On the 2nd challenge day, 3 ml of the same lung homogenate diluted up to $10^3$ CCU/ml was injected into each nostril. Concomitantly, intercurrent infections occurred after vaccination since *H. parasuis* was isolated in sentinel piglets (before challenge) and in controls and at the end of the study also in vaccinated piglets.

Due to intercurrent infections, including *M. hyorhinis* and antimicrobial treatment the study was considered invalid and no conclusion could be drawn.

In conclusion, in 3 out of 4 studies presented was demonstrated that the OOI is established at 3 weeks post vaccination, on the basis of reduction of lung lesions.

**Duration of immunity**

Four (4) studies were provided in order to demonstrate the DOI of the M. hyo part of Suvaxyn Circo+MH RTU.

The first study provided was conducted with the aim of demonstrating a DOI 10 weeks after vaccination in 3-week-old piglets.

A total of 114 crossbred pigs were enrolled in this study. Two (2) groups of crossbred piglets (38 animals each) were vaccinated at 3 weeks of age with two different vaccines containing the M. hyo antigen respectively at low potencies (M. hyo RP of 1.48 and RP of 1.00) and compared to a third group of control pigs treated with a vaccine containing only the PCV2 antigen. The 3 groups were challenged 7 weeks post vaccination on 2 consecutive days by IN and intratracheal route. *Bordetella* was isolated in sentinel animals before challenge but no circulation of M. hyo was detected and piglets were M. hyo seronegative.

The percentage of lung lesions (the mitigated fraction) was statistically significant reduced in the 2 vaccinated groups 4 weeks after the challenge.

In conclusion, DOI of 10 weeks was demonstrated but this DOI does not cover the entire fattening period of the animals.

The second study provided was conducted with the aim of establish the DOI at 19 weeks after vaccination in fattening pigs of 3 weeks of age. A total of 50 crossbred piglets were enrolled in the study. One (1) group of 20 pigs was treated with a vaccine at low potency (M. hyo of RP 0.96, i.e. below the minimum release potency) and 1 control group of 20 pigs was treated with a vaccine with only the PCV2 antigen at 3-week-old. The two groups were challenged 19 weeks later (at 22 weeks) on 2 consecutive days by the intratracheal route. A group of 10 pigs was left untreated as sentinels.

Despite intercurrent infections by *E. rhusiopathiae* and antimicrobial treatment the study was considered valid as the challenge met its validation criteria: 11% of control animals reported lung lesions and antibody responses indicated that exposure to the vaccine was similar than in other studies although no

significant differences was reported in the mean percentage of lung lesions in the vaccinated group (10%) and in the growth of the pigs when compared to the control group.

In conclusion, results from this study did not demonstrate the DOI against M. hyo for a period of 19 weeks after vaccination since no significant difference in lung lesions was shown between vaccinated pigs and controls.

The third study provided was designed with the aim of establishing the DOI for M. hyo at 23 weeks in finishing pigs of 3 weeks of age. The study had the same design as the previous study presented but pigs were challenged at 26 weeks of age (i.e. 23 weeks after vaccination). Circulation of M. hyo was detected in two piglets (one in the control and one in the sentinel group) before the challenge. The study was considered valid as all validity criteria were met. Percentages of lung lesion at the end of the study were not significantly different between study groups.

In conclusion, results from this study did not demonstrate the DOI against M. hyo for the period of 23 weeks after vaccination.

The fourth study was provided aiming to measure the DOI 16 weeks after vaccination in finishing pigs of 3 weeks of age. One hundred and twenty (120) piglets were included in the study. Two groups of 30 piglets were treated IM with vaccines at different potency (M. hyo RP of 1.22 and of 1.9). An additional group of 30 piglets was left as control and treated with commercial vaccine (Circumvent® PCV-M G2). Piglets were challenged intratracheally by a lung homogenate with unknown M. hyo burden. Ten (10) piglets were kept as sentinel. Results from the control groups showed seroconversion in all pigs and lung lesions in 5.8% of them. These results confirm the validity of the challenge.

Results in the vaccinated pigs did not show significant differences modified in comparison with the control neither in the growth of the animals nor in M. hyo colonisation of their respiratory tract. The percentages of lung lesions were slightly decreased (from 5.8% in controls to 1.5%–2.8% in vaccinates) and no dose/response relation was noted.

In conclusion, on the basis of results shown, the DOI against M. hyo was demonstrated for a period of 16 weeks after vaccination.

**Maternally derived antibodies**

A study was provided with the aim to evaluate the potential interference of MDA against the efficacy of the M. hyo part of the vaccine. A total of 154 piglets at 19–22 days of age were enrolled in this study. One hundred and eight (108) pigs had a mean MDA level of about 1.6 S/P by ELISA (similar antibodies level reached after experimental challenge in the efficacy studies). Two (2) groups of 36 MDA+ piglets were treated with vaccines containing M. hyo antigen at different low potencies (RP 1.09 and 1.52). One (1) group or 36 MDA negative (MDA-) piglets was vaccinated with the vaccine containing M. hyo antigen at potency of RP of 1.09 and 1 group of MDA+ pigs was vaccinated only with the PCV2 component of the vaccine. Ten (10) pigs were kept untreated as sentinels. Pigs were challenged 10 weeks post vaccination, when the level of MDA in 80% of the piglets was lower than 0.3 S/P ratio. The challenge met the validity criteria set by the applicant and this is considered acceptable and there was no indication of M. hyo circulation before challenge.

Results from groups treated with the low potency vaccines showed lower means of lung lesions of 4.8% in MDA+ and 7.2% in MDA- pigs. This difference was not statistically significant however, it should be considered that in the group of MDA- vaccinated pigs, the percentage of lung lesions was unusually variable and number of animals (36) too small to make any difference statistically significant (standard deviation value of 6.53). However, since the number of pigs used was compliant with Eur. Ph. the study is considered acceptable. Results from the group of the MDA+ piglets treated with the vaccine at higher

strength of (RP of 1.52, which is the minimum potency in the final formulation of the vaccine) showed a decreased percentage of lung lesions compared to MDA+ controls, however M. hyo load in their lungs was similar.

In conclusion, the study demonstrated that MDA, at levels routinely found in conventional piglets, do not interfere with development of immunity after vaccination. Therefore no specific warning in the SPC is necessary.

## *Field trials with PCV2 and M. hyo*

Three (3) GCP-compliant field studies were conducted in 3 commercial pig farms in the Netherlands with the aim to evaluate the efficacy of Suvaxyn Circo+MH RTU.

In the first field study provided a total of 130 conventional 21-day-old piglets were enrolled. Circulation of PCV2 and M. hyo was confirmed by serology before vaccination. One (1) group of 65 pigs was treated with an intermediate potent vaccine (PCV2 RP of 3.4 and M. hyo RP of 2.4) and one group of 65 piglets was left as control and administered with saline solution. No PCV2 or M. hyo seroconversion was observed after vaccination. A natural infection with PCV2 occurred after day 118 of the study. This was confirmed by the occurrence of PCV2 viraemia and faecal shedding in the control group as well as seroconversion in both the control and the vaccinated group however, PCV2 viraemia was significantly lower in the vaccinated group.

No difference in PCV2 shedding, lung lesions, mortality and percentage of animals receiving an antibiotic treatment during the study period was observed between vaccinates and controls. Growth was similar until day 89, thus before the PCV2 natural infection was detected, between the groups. At the end of the observation period (day 165), vaccinated pigs showed an average increase of 4.4 kg of weight gain compared to controls. This difference was statistically significant. Lung lesion scores at slaughter were low. The difference was not statistically significant.

In conclusion, results from this study showed that Suvaxyn Circo+MH RTU was able to significantly reduce PCV2 viraemia but not to reduce M. hyo associated lung lesions.

In the second field study provided, 399 conventional pigs 19–24 day of age were enrolled. Two hundred (200) piglets were vaccinated by IM injection with a low potency vaccine (PCV2 RP of 1.42 and M. hyo RP of 1.16), a further 200 piglets were kept as controls and administered saline solution. The finishing farm presented a history of PCV systemic disease and natural circulation of PCV2 happened between day 89 and 119.

Results from vaccinated pigs showed decreased PCV2 viraemia compared to the control group but not lower PCV2 faecal shedding. Zootechnical parameters, such as growth and mortality, were similar despite the antimicrobial treatment in both groups.

In conclusion, results from this study showed that there was no indication of M. hyo circulation due to the weak challenge and the percentage of lung lesions was very low in both groups. No statistical difference in vaccinated pigs and controls were observed. No conclusion can thus be drawn at the level of M. hyo field efficacy.

In the third field study provided, a total of 130 19–23-day-old piglets was enrolled. A group of 65 piglets was treated with a vaccine with high potency (PCV2 RP of 3.4 and M. hyo RP of 2.4) and a group of 65 piglets was left unvaccinated as control and treated with saline solution. The farm reported a history of M. hyo lesions at slaughter however during the field study no M. hyo infection circulated. On the other hand, circulation of PCV2 was detected serologically and viraemia rose from the start of the study.

Results from vaccinated piglets showed statistically significant reduction in viral load in blood during PCV2 infections; however, neither significant reduction in shedding nor decrease of clinical sign and improvement of zootechnical parameters (such as increased weight gain, mortality) was shown. No M. hyo infection occurred during the trial so no conclusion can be drawn at the level of efficacy under field conditions of the M. hyo part of the vaccine.

## *Conclusions on field trials regarding PCV2*

PCV2 infections occurred in the each field study at different times. The field studies demonstrated that the vaccine is efficacious in decreasing PCV2 viraemia regardless of the potency of the vaccine. Lesions and PCV2 load in the lymphoid organs were not investigated in the field studies. Only a few animals died during the studies, and there was no difference between the percentages of piglets with PCV2 positive lesions. Regarding to the clinical efficacy, the impact on the growth was not considered clinically relevant; the weight gain was statistically significantly improved only when the PCV2 infection was present at a late stage of the life cycle (about 1 week before slaughtering). In fact, no-positive impact on weight gain was shown when the PCV2 natural infection occurred in earlier stages of the life, i.e. 9 weeks before slaughter or 13 weeks before slaughter. Furthermore, vaccination did not shown to significantly decrease PCV2 faecal shedding. In conclusion, the 3 field trials supported only the decrease of a PCV2 viraemia.

## *Conclusions on field trials regarding Mycoplasma*

Of the 3 field studies, one was specifically designed to test M. hyo protection. However, M. hyo infection was not observed in any of the 3 studies as none of the piglets seroconverted and there was no significant level of lung lesions recorded at slaughter (0.6% to 1.8% lung lesions in control piglets). Therefore these field studies were inconclusive regarding the potential for confirmation of efficacy against M. hyo.

## *Overall conclusion on efficacy*

**PCV2**

The claims that the vaccine reduces viral load in blood and lymphoid tissues and faecal shedding caused by infection with PCV2 from 3 weeks after vaccination have been satisfactorily supported by laboratory and field studies.

The OOI was demonstrated in 6-week-old piglets, 3 weeks after vaccination.

The DOI was demonstrated at 23 weeks post vaccination.

In presence of MDA representative of field conditions the vaccine met the claims of reduction of viraemia, viral load in lymphoid organs and virus faecal shedding.

**M. hyo**

It can be concluded that vaccination is able to reduce the lung lesions caused by M. hyo under laboratory conditions. From the studies presented the OOI against M. hyo was demonstrated on the basis that the reduction of lung lesions was demonstrated at 3 weeks after challenge.

In conclusion, the DOI for the reduction of lung lesions set at 16 weeks.

Importantly no dose-response relation between the amount of antigen in the vaccine and the reduction of lesions can be drawn from the submitted studies. Overall, except in one study, the mycoplasma burden did not decrease in any of the samples or by any method chosen. In addition, the only clinical parameter monitored, the live weight gain, did not improved.

The 3 field studies provided were inconclusive because M. hyo infection never occurred during their course. In one study concurrent infections raised the percentage of lesion up to 45% and the percentage of lesions was reduced by 50% in vaccinated animals. These studies were inconsistent to demonstrate a clear reduction of lung lesions. The study design and specifically the challenge changed in the different studies.

The claims were met in the presence of MDA levels representative of field conditions at vaccination.

# Part 5 – Benefit-risk assessment

## *Introduction*

Suvaxyn Circo+MH RTU is an inactivated vaccine intended for prophylactic immunisation of pigs for fattening against porcine circovirus disease and porcine enzootic pneumonia.

Suvaxyn Circo+MH RTU is a bivalent vaccine, containing inactivated *Mycoplasma hyopneumoniae* and inactivated chimeric porcine circovirus type 1–type 2 (cPCV1-2).

The product is indicated for the active immunisation of pigs from 3 weeks of age against PCV2 to reduce viral load in blood and lymphoid tissues, faecal shedding caused by infection with PCV2 and for active immunisation of pigs over the age of 3 weeks against M. hyo to reduce lung lesions caused by infection with M. hyo.

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

## *Benefit assessment*

### Direct therapeutic benefit

In well-conducted laboratory studies the vaccine was shown to induce active immunisation of pigs against PCV2 in order to reduce viral load in blood and lymphoid tissues and faecal shedding caused by infection with PCV2. The product was shown to have an OOI at 3 weeks after vaccination with DOI of 23 weeks after vaccination against PCV2.

Efficacy was demonstrated against M. hyo regarding the claim of reduction of lung lesions. The vaccine was shown to have an OOI at 3 weeks after vaccination with a DOI of 16 weeks after vaccination against M. hyo.

The efficacy of the vaccine was adequately confirmed in the presence of MDA against both PCV2 and M. hyo.

### Additional benefits

None identified.

### Risk assessment

Main potential risks have been identified as follows:

*Quality:*

The formulation and manufacture of Suvaxyn Circo+MH RTU is well described and specifications set will ensure that product of consistent quality will be produced provided that the condition is fulfilled. At

present, the introduction of a pre-filtration step in the M. hyo manufacturing process has been considered necessary to lower the bioburden level before the final (sterile) filtration step and has been set as a condition to the marketing authorisation.

*For the target animals:*

Administration of Suvaxyn Circo+MH RTU in accordance with SPC recommendations is generally well tolerated in the target animal. In laboratory and field studies a transient increase in rectal temperature was frequently observed. Local reactions of maximum 2 cm in diameter were observed which disappeared within 2 days after vaccination. The vaccination did not negatively impact the daily weight gain until the end of the observation period. Immediate mild hypersensitivity-like reactions, resulting in transient clinical signs such as vomiting, diarrhea or depression, may occur uncommonly after vaccination and this is adequately reflected in the SPC.

*For the user:*

The CVMP concluded that the user safety for this product is acceptable when used as recommended and taking into account the safety advice in the SPC.

*For the environment:*

The product is not expected to pose a risk to the environment when used according to SPC.

*For the consumer:*

Residue studies are not required. The withdrawal period is set at zero days.

### *Risk management or mitigation measures*

Appropriate information has been included in the SPC to inform on the potential risks of this product relevant to the target animal, user, consumer, and the environment and to provide advice on how to prevent or reduce these risks. A condition to the marketing authorisation was considered necessary regarding the introduction of a pre-filtration step before the sterile filtration during the M. hyo antigen production process in order to reduce the bioburden before the sterile filtration step.

## 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 effectively induce active immunisation in pigs from 3 weeks of age against porcine circovirus type 2 (PCV2) to reduce viral load in blood and lymphoid tissues and faecal shedding caused by infection with PCV2 and active immunisation of pigs from 3 weeks of age against *Mycoplasma hyopneumoniae* to reduce lung lesions caused by infection with *M. hyopneumoniae*. The product was shown to have an OOI at 3 weeks after vaccination against PCV2 and M. hyo and a DOI of 23 weeks after vaccination against PCV2 and a DOI of 16 weeks after vaccination against M. hyo.

The formulation and manufacture of Suvaxyn Circo+MH RTU is adequately described and specifications set will ensure that product of consistent quality will be produced.

However, in order to decrease the bioburden level during the M. hyo antigen production process the introduction of a pre-filtration step was required before the sterile filtration step and it has been set as a condition to the marketing authorisation.

Suvaxyn Circo+MH RTU is well tolerated by the target animals and presents an acceptable risk for users and the environment when used as recommended and appropriate warnings have been included in the

SPC. The withdrawal period is set at zero days.

## Conclusion on benefit-risk balance

The overall benefit-risk evaluation for the product is deemed positive with a sufficiently clear and complete SPC and product literature.

## *Conclusion*

Based on the CVMP review of the data on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the application for Suvaxyn Circo+MH RTU 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).

Based on the CVMP review of the data on quality, safety and efficacy, the CVMP recommends the granting of the marketing authorisation for Suvaxyn Circo+MH RTU.