SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

Circovac is an adjuvanted vaccine containing inactivated porcine circovirus type 2 (PCV2) antigens. The vaccine is intended for the active immunization of sows and gilts with the aim to stimulate a specific immune response against PCV2. Passive immunity is transferred to piglets born from vaccinated pigs via the consumption of colostrum containing protective antibodies. The Applicant for this veterinary medicinal product is Merial S.A.S.

The complex nature of PCV2 infection is widely recognised. The presence of typical microscopic lesions (e.g. lymphocyte depletion) in lymph nodes and the presence of PCV2 within these lesions are considered to be the most reliable correlates for PCV2 infection under experimental conditions. A correlation between antibody titre against PCV2 and protection from PCV2 associated disease has also been demonstrated.

A number of co-factors, such as infectious agents, may increase the severity of PCV2 infection, resulting in various disease syndromes (collectively named PCV2 associated diseases or PCVD) characterised by different clinical expression forms.

The rationale behind the design of Circovac is to provide newborn piglets with an earlier immunity against PCV2 infection. Early infection by PCV2 is assumed to result in the most severe expression of PCV2 associated diseases, such as Post-Weaning Multi-Systemic Wasting Syndrome (PMWS). Vaccination of dams is aimed at improving and increasing maternal immunity thus allowing the transfer to progeny of a more homogeneous and higher level of passively acquired immunity.

The dose of the vaccine is 2 ml and it is administered by deep intramuscular injection.

The basic vaccination schedule for gilts is: initially two doses at an interval of 3 to 4 weeks, at least 2 weeks before mating; one further injection must then be given at least 2 weeks before farrowing.

The basic vaccination schedule for previously unvaccinated sows is: initially two doses at an interval of 3 to 4 weeks, with the last dose being given at least 2 weeks before farrowing.

The revaccination schedule consists of one injection during each gestation, at least 2 to 4 weeks before farrowing.

The benefits of Circovac are its ability to reduce typical lesions in the lymphoid tissues of piglets caused by PCV2 and as an aid to reduce mortality associated with PCV2 infection.

The most common side effects are slight and transient local reactions in vaccinated animals (sows and gilts), mainly swelling and redness, and in some cases oedema, which usually resolve within 4 days following injection. An increase in rectal temperature can also occur within the 2 days following injection. In rare cases, slight apathy or reduction in appetite may be observed, but these should resolve spontaneously.

The approved indication is: “Passive immunisation of piglets via the colostrum, after active immunisation of sows and gilts, to reduce lesions in lymphoid tissues associated with PCV2 infection and as an aid to reduce PCV2-linked mortality.”

The duration of immunity is up to 5 weeks after the transfer of maternally derived anti-PCV2 immunity to the piglets through colostrum intake.

2. QUALITY ASSESSMENT

Composition

Circovac is a liquid vaccine and the product is presented as two vials. The first contains an aqueous suspension, the active ingredient consisting of the inactivated PCV2 antigen diluted in phosphate buffer solution (PBS), and thiomersal as preservative. The second vial contains an oil-in-water emulsion consisting of light paraffin oil as adjuvant, a mixture of surfactants as oily excipients (such as polysorbate 80, sorbitan oleate, and polysorbate 85), and thiomersal as preservative. The suspension and emulsion are mixed immediately prior to use and the reconstituted vaccine is used within 3 hours.

Circovac contains at least 2.1 $\log_{10}$ ELISA units of inactivated PCV2 antigen in each 2 ml dose.

Containers

Two sizes (of primary packaging) are available, the smaller containing sufficient vaccine (once the two vials are mixed) for 5 doses and the larger containing 25 doses. The 5 dose presentation comprises 5 ml vials containing at least 3.34 ml of the antigen suspension and 10 ml vials containing at least 6.66 ml of the emulsion adjuvant. The 25 dose presentation comprises 20/24 ml vials containing at least 16.67 ml of the antigen suspension and 50/58 ml vials containing at least 33.33 ml of the emulsion adjuvant. Both the antigen suspension and adjuvant emulsion are filled into Type I glass vials sealed with rubber stoppers and closed with aluminium overseals/caps.

Development Pharmaceutics

The product was developed in response to the significant impact in Europe of PMWS in piglets.

Currently no vaccine is authorised in the EU against any PCV2 associated diseases and, specifically, against PMWS. However, a total number of 1.7 million doses of Circovac are reported to have been used under special provisions, for vaccination campaigns for gilts and sows carried out in both France and Germany.

Circovac is produced and tested using well standardised and acknowledged processes.

The choice for the field isolate of PCV2 used for the production of the vaccine (the 1010 PCV2 strain was isolated from a pig suffering from PMWS in Canada in 1997), is justified by available literature supporting the homogeneity of PCV2 strains isolated worldwide and the similarity of biological characteristics among them. The choice of the development of an inactivated vaccine is justified by minimising the risks for target animals, e.g., pregnant sows and gilts. Although the minimum antigen content had been set at 2.1 $\log_{10}$ ELISA units/dose, in order to ensure a satisfactory margin, the formulation target after reconstitution was set at 2.4 $\log_{10}$ ELISA units/dose, which corresponds to significantly higher antigen content compared to the minimum one. The selected formulation is compatible with the need to ensure an adequate immune response whilst ensuring an acceptable level of safety in the target species. The addition of thiomersal is justified by the multidose presentation of the vaccine.

Information on the key stages of production of both the antigenic suspension and the adjuvant emulsion is presented, as well as on all the different stages of the manufacturing processes. Evidence of compliance with the manufacturing process and also of batch-to-batch consistency in the finished product is provided. Data and information are provided in relation to the main characteristics of starting materials, certified or tested to ensure the quality of the product and compliance with current EU requirements. Specific and commonly applied tests are carried out during production, and, as no specific European Pharmacopoeia monograph for PCV2 is currently available, testing of finished product relies on general EU/EP monographs or guidelines.

The use of a PK15 cell line harbouring porcine endogenous retrovirus (PERV) sequences known to be tumourigenic for nude mice cells was considered acceptable as assurances were provided that the

manufacturing process allows control of any potentially undetectable risk of retrovirus contamination in the final product. Data provided showed that the overall risk posed by infectious retrovirus particles that could potentially be released from the PK15 cell line is negligible and consequently the CVMP concluded that safety of the PK15 cell line for vaccine production was acceptable.

During development, the product was changed from a ready-to-use 1 vial presentation to the final formulation, a 2 vial presentation (of antigen suspension in one vial and adjuvant emulsion in the other). Even though most of the primary safety and efficacy studies were conducted with the 1 vial presentation, bioequivalence studies support this change and the stability of the reconstituted vaccine (under the conditions of use) was proven.

Filling overages are specified and justified for the different vial sizes.

Method of manufacture

All production steps are performed in accordance with GMP. A flow chart of the method of preparation of the active substance, the vaccine suspension and the adjuvant emulsion is presented and the methods are described in detail. (The method of production of the single/ready-to-use vial that was used in the initial studies is also presented.)

All manufacturing operations are conducted in closed circuits, the connections in which are sterilised by steam (and such sterilisation operations comply with the requirements of the European Pharmacopoeia). The critical stages of manufacture have been identified.

Preparation of the vaccine suspension:

The manufacture vaccine suspension, including purification of PCV2 antigen, is fully described and details of the control tests performed are provided. Following dissociation by trypsin and amplification steps performed on microcarriers in stainless steel vessels, cells are infected with the working seed virus and allowed to multiply simultaneously under appropriate conditions of temperature and pH. After the culture is terminated the harvest is treated in order to rupture the cells. Cell debris is then removed by centrifugation and the virus harvest stored at an appropriate temperature. A two step inactivation process is carried out under detailed conditions of temperature, pH and timing by adding pre-determined concentrations of betapropriolactone (BPL) to the purified virus harvest. Monitoring of pre-inactivation titres is routinely carried out, and the CVMP were of the opinion that such a practice demonstrates adequate control of the production process of the virus suspension. The inactivated harvest is then concentrated by ultrafiltration. The period of time the concentrated active ingredient is stored before further processing has been stated and justified.

For the preparation of final vaccine suspension, concentrated PCV2 antigen and thiomersal are added to phosphate buffer solution sterilised by steam prior to use, and mixed. Blending takes place over one working day.

The vaccine is blended using a fixed target content of inactivated antigen corresponding to 2.58 $\log_{10}$ ELISA units/ml bulk in order to guarantee at least 2.1 $\log_{10}$ ELISA units per 2 ml dose over the whole period of validity of the vaccine.

Preparation of the adjuvant emulsion:

The manufacture of the adjuvant emulsion and the control tests performed are described in detail.

The oily phase is produced by mixing the sorbitan oleate, the polysorbate 85 and the light liquid paraffin, heating this mixture and sterilizing it by filtration. The oily phase is transferred to the emulsification vessel, cooled and then the aqueous phase added.

The aqueous phase is manufactured by mixing the polysorbate 80 with phosphate buffer solution; sterilizing that by steam; adding thiomersal; and cooling. The aqueous phase is then transferred to the

emulsification vessel containing the oily phase. The resultant emulsion is then cooled and stored until filling. The period of storage before filling can be up to 2 weeks, which has been justified.

Validation studies
Two studies are presented in order to validate the process of retrovirus inactivation, in case of undetected release of infectious particles during Circovac vaccine manufacturing process. Both studies followed the principles set in the EU Note for Guidance CPMP/BWP/268/95 on design, contribution and interpretation of studies validating the inactivation and removal of virus.

Inactivation kinetics for the manufacturing process are given. The first of such study investigated the inactivation kinetics in a non-concentrated bulk viral harvest, and the second at a concentrated bulk harvest. Both studies support the specified maximum pre-inactivation titre of approximately 6.4 $\log_{10}$ CCID 50/ml.

CONTROL OF STARTING MATERIALS
The following starting materials listed in a Pharmacopoeia comply with the Ph.Eur. requirements:
Gentamicin sulphate
Sodium hydrogen carbonate
Trisaminomethane
Sodium hydroxide
Hydrochloric acid
Ester of fatty acids and of ethoxylated polyols
Ester of fatty acids and of polyols
Thiomersal
Sodium chloride
Sodium phosphate dehydrate
Potassium dihydrogen phosphate
Water for injection

The PCV2 isolate 1010 used for vaccine production originates from a PMWS affected pig in Canada in 1997. This isolate was chosen as the prototype vaccine strain because of lack of evidence of genetic and biological differences among PCV2 isolates described worldwide.

The Master Seed Virus (MSV) was obtained after culturing the strain 1010 of PCV2. Details of the identification and control of the MSV are provided. Purity of the MSV strain is demonstrated according to Ph.Eur. monograph 0062 and the relevant EU guidelines.

The Working Seed Virus (WSV) is stated to correspond to the 4th passage (at most) from the MSV and a certificate of analysis is provided for a batch of working seed lot. Batches of active ingredient consist of the 5th passage (at most) from the MSV (at most one passage further from the WSV). Details of the WSV controls, storage conditions, descriptions of the different phases of production of the active ingredients (culture, harvest, treatment, inactivation and concentration) and examples of preparation of three consecutive batches are provided and are satisfactory.

Testing of both the MSV and WSV complies with the PhEur requirements.

Cell substrate: PK 15 cell line:

Original PK15 cell stocks were obtained at passage 166 from Dr. Ellis laboratory at the University of Saskatchewan, Canada (as well as the 1010 PCV2 strain). Four passages were completed to define the Master Cell Bank (MCB = 170th passage, stored in liquid nitrogen and identified by a specific code recalling the number of passages and the date of constitution).

The Working Cell Bank (WCB) is prepared from one ampoule of MCB. Cells at the sixth passage from the MCB constitute the WCB. A seed lot is used. Techniques and certificate of analysis have been provided for the established MCB and for the WCB. The purities of the MCB, of MCB+20 passages, and of seed lot of PK15 were adequately demonstrated in accordance with the EU guideline

on extraneous agents (III/3427/93) and with the Ph.Eur. monograph 0062. The origin and karyotype of the cells were also confirmed for MCB and MCB + 20 passages.

Testing complies with the PhEur requirements for:

- *Calf serum*
- *Donor serum*
- *Foetal calf serum*
- *Trypsin C*

Certificates of Analysis and details of countries of origin of the animals from which the starting material listed above originated were provided.

The bovine serum is gamma irradiated with a minimum dose of 35 KGy which is considered high enough to inactivate any residual bovine pestivirus, and this is in accordance with the CVMP guideline on bovine serum. The trypsin is irradiated using the same dose.

The following starting materials of non-biological origin are not in a Pharmacopoeia but nevertheless meet the relevant general Ph.Eur. requirements and appropriate specifications are provided:

- Betapropriolactone (BPL)
- Polysorbate 85
- Light liquid paraffin

The microcarrier used is composed of a dextran substance.

Information is provided reassurance regarding the in-house preparation and quality of the following media.

- PK15 medium
- PCV2 medium

Packaging

The vials used for both the suspension and emulsion are colourless sterilised (by dry heat) glass Type I vials which are sealed with rubber stoppers (butyl rubber for the suspension vials and nitryl rubber for the emulsion vials) and then closed with aluminium overseals/caps. Both types of the stoppers are washed, silicon coated (the silicon meets the requirements of the Ph.Eur. monograph), and then sterilised, by autoclaving at 121°C for at least 15 minutes in compliance with the Ph.Eur. requirements. Satisfactory specifications and certificates of analysis were provided.

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

The TSE-Risk assessment for Circovac according to Commission Directive 1999/104/EC and Note for Guidance EMEA/410/01-Rev. 2 includes the following starting materials of animal origin used in the production of this vaccine:

- Foetal calf serum
- Calf serum and donor calf serum
- PCV2 culture medium is prepared using pepticase, beef extract and bovine peptone and includes human insulin
- Trypsin

Where relevant, statements from the manufacturers with respect to the source of the materials and copies of EDQM Certificates of Suitability are provided and risk assessments for all biological components are given. The country of origin of any material derived from milk was also stated in compliance with the TSE requirements. These starting materials therefore are considered to constitute a negligible risk of contamination with TSE agents.

The risk assessments for cell lines and viral seeds were also provided and demonstrated TSE compliance.

In summary, the Committee agreed that the starting materials of animal origin used in the production of the final product comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC and that the TSE risk for this product can be regarded as negligible.

Control tests during production

Descriptions of the control tests performed during the production of the suspension and emulsion (such as temperatures, checks for sterilising filter integrity, sterilisation cycle monitoring, etc) are given, for both the vial sizes. These tests are satisfactory to control the production processes for each of the vials and to ensure consistency of production. The checks also cover the filling and packaging processes.

CONTROL TESTS ON THE FINISHED PRODUCT

Specifications are provided for both the emulsion and suspension vials, and for both vial sizes of the product.

The methods used to control the PCV2 suspension vial contents (visual appearance, volume, pH, antigen quantification, thiomersal content, sterility and safety tests) are described and have been satisfactorily validated. The specifications proposed are appropriate to control the quality of the antigen suspension vial.

Inactivation test on the finished antigen suspension: a commitment has been made to implement this if the revision of the technical annex to the Directive retains this test.

The identification and assay of the active substance is performed by measuring the ORF2 protein using an ELISA method. Justification of how the lower limit in the specification has been set in relation to efficacy data is provided. The use of the ORF2 protein to characterize the viral content of the vaccine is justified and evidence for the relevance of ORF2 protein is provided (in the form of a series of references relating to the organization of PCV genome). The use of an *in vitro* potency test has been accepted by the CVMP. The ELISA has been suitably validated and the potency test as a whole has also been validated to assure that the minimum level of antigen content is efficacious.

The safety test is performed on each batch and full details are given. With regards to the temperature effects, the mean rectal temperature increase after vaccination of pigs in comparison to rectal temperature measured at day 0 must be inferior to or equal to 2°C with a return to normal after vaccination. Only moderate signs linked to hyperthermia are accepted. These signs must decrease at day 1 and disappear at day 2 after vaccination.

The methods used to control the adjuvant emulsion vial contents (visual appearance, volume, pH, density, viscosity, thiomersal content, safety (after reconstitution), sterility) are described and have been satisfactorily validated. The specifications proposed are appropriate to control the quality of the adjuvant emulsion vial however, it was considered that a test to quantify fatty acids should be conducted and a commitment to this effect has been made. A commitment has been made to implement tests on the final reconstituted product (that is on the 2 vials mixed as for use). These tests should include the physical tests, viscosity and density and also pH and thiomersal. The specifications should be those demonstrated for the 1 vial presentation.

Results from the analysis of three batches of the finishes product are provided which demonstrate that the manufacturing process produces product of the defined quality and that there is consistency between the batches. However, a commitment has been made for the submission of three batch protocols for the diluent/emulsion vials for the 5 dose presentation when these become available.

STABILITY

Stability of the finished product:

Stability data have been provided for 5-dose presentation which demonstrate that a 12 months shelf-life when stored at 5°C is justified. A complete 15 month stability report for the 25-dose presentation is also provided.
Satisfactory preservative efficacy data have been provided, for both the suspension and the emulsion.
The storage precautions for the SPC were agreed as: "Store and transport refrigerated (2 °C – 8 °C). Do not freeze. Store in the original package in order to protect from light."

Stability of the reconstituted product:

Data have been provided for the reconstituted vaccine and these support the instructions in the SPC and other product information that, once reconstituted, the vaccine should be used within 3 hours.

GMO - Not applicable.

OVERALL CONCLUSION ON QUALITY

The analytical dossier is well described. Documentation and specifications reflecting the actual manufacturing and testing processes for production of the PCV2 antigen component were provided in detail.

The methods of manufacture for both vials of the product are well described and the in-process controls detailed in full. The compliance of starting materials of animal origin used during production with the requirements of the Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary products was shown.

The specifications for both vials of the products and also for the reconstituted product (as ready for use) are comprehensive and adequate to control the quality of the product. Appropriate validation data were provided.

Batch analysis data demonstrate consistency of manufacture of the finished product.

Based on the stability data provided, a shelf-life of 12 months for the finished product (when stored refrigerated) is justified. It has also been demonstrated that, once reconstituted, the product may be used for up to 3 hours. The SPC and other product information include all the necessary information regarding storage and reconstitution of the product.

The TSE risk for this product can be regarded as negligible.

3. SAFETY ASSESSMENT AND RESIDUES

Safety studies have been carried out in the target species, the pig, in three laboratory trials and two field trials, which were generally in compliance with the current requirements. Additional data were submitted from use in the field of the vaccine in Germany and France. The following safety issues were assessed:

- safety of a single dose, repeated dose and overdose
- safety in the field

The composition of all the batches of vaccine used in the safety studies are briefly described. Some of the studies were performed with the original ready-to use/one vial presentation of the vaccine, but more recent studies were performed with the commercial (two vial, suspension and emulsion) formulation.

In the laboratory studies, the batches used were of maximum antigen content (2.7 $\log_{10}$ ELISA units per 2 ml dose).

The field trials were performed with batches of maximum antigen content. The additional data from use in the field in 22 French farms and 38 German farms was obtained using the product with an antigen content of 2.4 $\log_{10}$ ELISA units per 2 ml dose.

A. SAFETY ASSESSMENT

LABORATORY TESTS

Three laboratory studies in SPF (specific pathogen free) pigs were performed.

The first study investigated the safety of one dose and the repeated administration of an overdose (double dose) in SPF non-pregnant gilts. The second study investigated the safety of one overdose dose and the repeated administration of single doses in SPF pregnant gilts. The third study investigated the safety of repeated overdoses in SPF pregnant gilts.

In these laboratory safety studies, the reproductive performance of the SPF gilts was also examined.

In all the safety trials, vaccinated animals underwent thorough clinical monitoring. Individual body temperature, general body condition, local reaction monitoring, lesion scoring at slaughter (with specific recording of the lesions observed at injection sites and on the local lymph nodes) were recorded, and then followed by histological examination.

In the two studies involving pregnant gilts, their progeny were monitored for zootechnical criteria and weight gain.

Details of the batches used in all the safety studies were reported.

Safety of the administration of one dose and the repeated administration of a double dose

The objective of this GLP compliant study was to investigate the safety of Circovac for gilts and their progeny after intramuscular administration to gilts.

Safety of the administration of one dose:

The vaccine batch used was the 1 vial (ready-to-use) presentation. This was fully characterised and contained twice the standard antigen content (2.7 $\log_{10}$ EU/dose).

Pregnant SPF gilts were used in this study as SPF animals in the late stages of pregnancy were considered to mimic the worst scenario for vaccination. Three homogeneous groups of pregnant gilts (aged 51 to 70 weeks), were investigated:

- Group 1 acted as control and received two repeated administrations of 2 ml of placebo (physiological saline) by intramuscular injection in the left and right sides of the neck at 2 week intervals.
- Group 2 was the test group for the safety of one dose by intramuscular injection in the right side of the neck 14 days before the expected farrowing date.
- Group 3 was the test group for the safety of a double dose and repeated overdoses. This group received two repeated administrations of 4 ml of vaccine by intramuscular injection in the left and right hand sides of the neck at 2 week intervals.

The pregnant gilts underwent thorough clinical monitoring: individual body temperature, general body condition, local reaction monitoring, lesion scoring at slaughter (5 weeks after the last vaccination) (with specific recording of the lesions observed at injection sites and on the local lymph nodes) were recorded, and then followed by histological examination.

For each litter, data regarding farrowing date, characteristics of piglets at birth (e.g. consistency of litter, born alive, stillborn or mummified, sex, weight), number and weight of weaned piglets were recorded. Blood samples from the piglets were taken twice, at one and three weeks of age, to validate colostrum consumption. The piglets also underwent thorough clinical monitoring where appropriate.

In group 2, rectal temperature increased up to 39.3°C six hours after vaccination. Although the average increase of rectal temperature was limited to 0.6°C (from 38 to 38.6°C), in a few pigs the increase was higher (up to 1.5°C). Twenty four hours after vaccination the rectal temperature had returned to normal levels. Signs of depression, vomiting and loss of appetite or anorexia were recorded, but not at or near the time of vaccination. No other general symptoms were recorded in pigs vaccinated with one dose of Circovac.

The injection of one dose of Circovac appeared to have been well tolerated by pregnant gilts as far as general body conditions are concerned.

Six hours after vaccination the majority of the gilts presented induration at the injection site which lasted no more than two days, except in one gilt which still showed induration at day 26/27. Oedema was recorded in 8% of the gilts for four days after almost three weeks from vaccination. Redness was observed in two-thirds of the gilts one or two days after vaccination. In only one pig redness, lasting 2 days, was re-observed two weeks after vaccination. No heat and pain was ever recorded. When compared with the local reactions observed in control pigs following injection of physiological saline, a significant difference was recorded. Whereas no control gilts showed lesions at injection sites, almost all gilts in the test group presented different types of lesions varying from discolouration to presence of granuloma and fibrosis of tissues. The average volume of the lesions was about 20 cm^3, ranging from a minimum of 4 to a maximum of 64 cm^3. No involvement of local lymph nodes was recorded.

By excluding the gilts which appeared not to be pregnant and two gilts which gave birth to small litters (both explained by the poor reproductive performance of SPF animals), no effect of Circovac vaccination on the reproductive performances of the gilts was evident.

Gilts were free of antibody before enrolling and no seroconversion was observed in the controls. The antibody level in one week old piglets born to vaccinated gilts was significant, but by three weeks of age the levels of antibody can be considered negative.

This study was well performed and provided evidence that one dose of Circovac is generally well tolerated by pregnant gilts in which their general condition was satisfactory after vaccination, without any adverse impact on reproductive performance. Furthermore, vaccination with a single dose did not have any significant effect on the number of piglets born alive, remaining alive, or on the daily weight gain of surviving piglets.

Safety of the (repeated) administration of an overdose:

Group 3 mentioned in the previous section were studied to assess the safety of the administration of repeated overdoses. The same vaccine batch was used as in the previous section.

Gilts from group 3 received 4 ml (a double dose) of Circovac on the left side of the neck at day 0.

Control gilts (group 1) received 1 dose of physiological saline on the left side of the neck at day 0 and on the right side of the neck 14 days later (day 14).

The same parameters were examined.

The average increase of rectal temperatures after first and second vaccination appeared to be in line with the control group. Looking at individual results however, 6 hours after vaccination, an increase of rectal temperature up to 1.5°C was recorded in 15% of the gilts.

Six hours after the second vaccination with the product, the average increase of rectal temperature was already significant compared to the other groups (1.3 degrees, from 37.6 to 38.9), but individual data showed increase in temperature up to 2°C. In one-third of the gilts the increase was higher than 1.5 degrees. An increase up to 2.5 degrees was recorded by day two after vaccination in one gilt which never recovered: piglets born from this gilt (which had to be euthanased) died within 4 days after birth. Necropsy of the gilt attributed its illness to lameness and a gastric ulcer, and not to the vaccination.

After the second administration of a double dose of the vaccine, signs of depression and loss of appetite were recorded in half the gilts, but not near the time of vaccination. Only one isolated pig exhibited a reduction in appetite 2 days after injection. Vomiting was observed in one isolated pig one day after administration of the second double dose. The statistical analysis performed revealed no statistically significant difference among the three test groups. After the first administration of physiological saline, gilts in the control group did not generally present with any local reaction. After the first injection of a double dose of vaccine, however, redness (between day 1 to day 6) or moderate induration (lasting generally no more than two days) were recorded in two-thirds or half of the vaccinated gilts respectively. Local reactions were recorded in the control group after the second injection of placebo. 100% of gilts under test showed induration of moderate intensity that disappeared, in almost all cases, 8 days after injection. Three-quarters of the gilts showed oedema (1 to 11 days) after injection of the second overdose of vaccine and the same percentage of these gilts exhibited redness. In these cases the type of reactions was quite important. Although no pain was recorded, heat was present in three gilts on different days. Statistical analysis revealed a significant difference between the control and test groups. At necropsy, no control gilts showed lesions at the injection site, whereas all the gilts in the test group exhibited fibrosis or granuloma. The volume of the lesions was generally of medium size (up to 24 cm^3) but in rare cases the lesions were larger (up to 60 cm^3). In one isolated case, an abscess of 30 cm^3 was reported following the second injection of an overdose of the vaccine.

One-sixth of the gilts, in each of the control group and the group under test, were not pregnant. One of the control group gilts aborted 9 days after the first administration of placebo. One gilt had to be euthanased for ethical reasons because of arthritis and a deep gastric ulcer. The administration of repeated overdoses of Circovac had no effect on the number of piglets born alive, remaining alive, or on the daily weight gain of surviving piglets. The results, therefore, demonstrate that treatment of pregnant gilts with double and repeated double doses of the vaccine did not influence the reproductive performances of the animals.

As far as antibody response is concerned, high titres of antibody to PCV2 were present in gilts at farrowing and at slaughtering. A consistent amount of antibody was also present in piglets at 1 and 3 weeks of age.

Safety of one overdose and repeated administration of one dose in SPF non-pregnant gilts

The objective of this GLP study was to investigate the safety of an overdose of Circovac followed by the repeated administration of one dose, in 6 month old SPF non-pregnant gilts. The same vaccine batch was used as in the previous studies.

Six month old SPF non-pregnant gilts were randomly assigned into one of two groups:

- Group 1 acted as control and received an initial intramuscular injection of 4 ml of placebo (physiological saline) by intramuscular injection in the left side of the neck at day 0, followed by a second i.m. injection of 2 ml of placebo in the right hand side of the neck at day 21, then a third i.m. injection of placebo in the left buttock at day 42.
- Group 2 were the treatment group and received an initial intramuscular injection of 4 ml of Circovac by intramuscular injection in the left side of the neck at day 0, followed by a second i.m. injection of 2 ml of Circovac in the right hand side of the neck at day 21, then a third i.m. injection of Circovac in the left buttock at day 42.

Observations and investigations were performed in an almost identical manner to the previous study, up to day 70 (28 days after the 3rd injections) with additional blood testing at the start and end of the study. Necropsy was performed at day 71.

Rectal temperatures peaked 5 hours after the first and third injections (average = +1.0 and 1.5°C respectively) and 24 hours after the second injection (average = + 1.1°C). They returned back to normal 24 hours after the first and third injections and 48 hours later after the second injection. Temperatures were comparable in the control and vaccinated groups. The highest temperatures observed in gilts were ≤ 41.1°C. Increases of temperature were recorded of ≥ 1.5 in 30% of animals 5 hours after the first vaccination with an overdose, and in 40% of animals after an initial injection of a single dose.

No general reactions were observed in the gilts in the placebo group (group 1). General reactions in the treatment group were few and transient, mainly apathy and loss of appetite, although anorexia, vomiting and dyspnoea were less frequently observed. These effects were mostly recorded in the treatment group, and mainly after the third injection.

No local reactions were observed at any time in the gilts in the placebo group. Moderate induration, lasting one or two days, was observed in half of the vaccinated gilts (group 2) after the first injection. Redness was observed in 40% of the gilts 24 hours post-vaccination. Most vaccinated pigs presented with more pronounced induration after the second injection, and this lasted from 2 to 7 days. Redness (medium), lasting from 1 to 5 days, was also recorded in 70% of animals after the second injection. Oedema was recorded in 20% of vaccinated pigs. After the third vaccination no oedema was observed, whereas most pigs showed less marked induration (which lasted from 1 to 11 days). Redness was recorded in half the vaccinated gilts and this lasted from one to three days. No heat or pain was recorded.

No lesions were recorded in control pigs. Discolouration was recorded in most pigs from the treated group. An abscess (of moderate size) was exceptionally observed. Microscopical examination of the reactions at the injection sites revealed, in most cases, granuloma, necrosis and/or fibrosis.

At the beginning of the study all the animals were seronegative. Gilts in the control group remained seronegative at the end of the study. A strong seroconversion was recorded in all the treated gilts.

Safety of the repeated administration of a double dose in SPF pregnant gilts

The objective of this study was to assess the safety of three injections of a double dose (i.e. overdose) of Circovac administered in SPF pregnant gilts. In contrast to some of the earlier studies, the final formulation (2 vial presentation) was used.

Nine to twelve month old SPF pregnant gilts were assigned to one of two groups. Half were vaccinated with a double dose of the vaccine three times (at days 0, 21 & 42, equal to approximately 9, 6 and 3 weeks prior to farrowing) and half served as the controls and received the equivalent volume of normal saline as placebo.

Observations and investigations were performed in an almost identical manner to the previous studies.

Several parameters were examined in order to evaluate the safety of the vaccine: monitoring of general and local reactions (including rectal temperature); reproductive performance (number and growth of offspring). In addition, seroconversion to PCV2 after vaccination was also evaluated (blood samples were collected on D0 (prior to vaccination), D71 (after farrowing), and D92 (at weaning)).

General reactions mainly consisted of moderate vomiting and reduction in appetite/anorexia, both in the vaccinated and the control gilts. The results in both groups were similar for each period of observation, so no link with the vaccine was established. No statistically significant differences were recorded (e.g. entity and incidence of clinical signs).

Before the first injection, the average temperature of the vaccinated group was lower compared to the control group. Four hours after the first injection, an increase of temperature was observed in both groups (+1°C in the vaccinated group and +0.3 °C in the control group, the average rectal temperature reaching 38.5°C and 38.3°C respectively). From day 1, no major differences were observed between the two treatment groups. The average rectal temperature was similar as far as the second and third injection are concerned (after 4 or 5 hours: +0.4°C for the vaccinated group and +0.3°C for the control group after the second injection; +0.9°C for the vaccinated group and +0.4 °C for the control group after the third injection).

Local reactions consisted of swelling, redness, induration at/of injection sites. All vaccinated gilts showed a moderate swelling after each injection, appearing from 5 hours to one week following injection. After each vaccination, swelling lasted up to 9 days in average. The incidence of swelling in vaccinated pigs after first and second vaccination was statistically significant, while only a trend toward higher incidence was observed in vaccinated pigs after the third injection as compared with control pigs. The statistical analysis showed that the incidence of redness was significantly higher in the vaccinated group only after the first injection, while no difference was observed between the two treatment groups after second and third injections. The statistical analysis showed that the incidence of induration was not significantly different between the two treatment groups. The statistical analysis of total score of local reaction, performed only for the third injection, showed a significant different between the two groups.

The statistical analysis confirmed that both groups were not significantly different in relation to litter size, number of live piglets, number of weaned piglets. The statistical analysis confirmed that there was no significant difference between both groups in relation to the relative average daily weight of the piglets. Vaccinated and control gilts showed similar non specific lesions at necropsy (e.g. erosion of the gastric mucosa, gastric ulcer, discoloration of kidneys, bladder congestion) mainly related to the stress induced under the conditions of the study. At the end of the study, no external lesions were observed at visual inspection or palpation of the injection sites. After dissection, local discoloration of the muscular tissue, or presence of granuloma were observed. The histological analysis showed that the majority of the injection sites examined presented a moderate to severe granulomatous inflammatory reaction.. Signs of haemorrhage, fibrosis and/or necrosis were observed in some cases. No major differences were microscopically observed between the three injection sites for any of the pigs examined. From the first injection to weaning, the gilts of the control group remained seronegative towards PCV2. The vaccinated gilts seroconverted to very high ELISA antibody titres at farrowing and weaning. The piglets born to vaccinated gilts presented high antibody titres from farrowing to weaning while the piglets born from control gilts remained seronegative during all the observation period.

From the results obtained from this study, it is apparent that 3 injections of a double dose of Circovac administered intramuscularly in pregnant gilts, 9, 6 and 3 weeks before farrowing were well tolerated

as shown by the absence of treatment-related general reactions and the absence of impairment of the reproductive performances of the gilts. Transient and moderate local reactions associated with an underlying granulomatous inflammatory reaction were the adverse reactions mainly associated with the administration of the vaccine.

It is considered that the safety profile of the vaccine as described in the SPC of Circovac is adequately supported by the results obtained from this study.

Examination of reproductive performance

See the sections above. The vaccine is intended for administration to pregnant gilts and sows, before mating (gilts) or farrowing (gilts and sows).

As Circovac is intended for pregnant sows, it was essential to evaluate the possible impact on the reproductive performance. Overall, vaccination had no impact on the characteristics of the litters in the studies. Abortions were reported in 0.4% of animals in the laboratory and field trials. Although no definite link with the vaccine was established, a warning regarding the exceptional possibility of abortions was included in the SPC as a precaution.

Examination of immunological functions

The product does not contain any component for which an immunosuppressive effect may be suspected.

Interactions

No specific reference to such an issue is included. Gilts and sows receive a wide spectrum of vaccinations (e.g. against Aujeszky disease, *E. coli* and PRRSV). Although there has been speculation in the literature concerning the interaction of early PRRS vaccination with the onset of PMWS, adequate justification is provided for the absence of a study to investigate any interaction of circovirus and vaccination against PMWS with other prevalent causative agents of pig diseases.

FIELD STUDIES

Field study 1:

A controlled multicentre field trial was conducted by an independent organisation on conventional gilts and sows in 3 farrowing-to-finishing farms in France (Brittany) in order to evaluate the overall safety of the vaccine in conventional pregnant sows and gilts.

The trial was performed according to the principles of the guideline "Good clinical practice for the conduct of clinical trials for veterinary clinical products". The 1 vial presentation of Circovac (with a maximum titre of 2.7 $\log_{10}$ Elisa Units) was used.

Although no symptoms of PMWS (no wasting) in piglets or fattening pigs had been reported for any of the three farms, circulation of PCV2 in the area and in the herds was suspected, and indeed demonstrated by the strong seropositive results in pigs at selection and start of the trial.

Treatment group (Group A):
- Gilts – a dose of the vaccine was administered twice (the doses were given 3 weeks apart) before mating and a third dose was given two weeks before the expected date of farrowing.
- Sows were injected with a dose of vaccine 2 weeks before the expected date of farrowing.

Control group (Group B):
- The gilts/sows received an injection of saline solution (using the same schedule as the vaccinated animals).

After farrowing, reproduction performance (number of born alive, stillborn, mummified) was recorded for each gilt/sow. After weaning, the weaning-fertile mating interval and the number of weaning piglets were recorded. In each case vaccinated pregnant gilts and sows were compared to the negative controls (injected using the same schedule with a saline placebo).

The parameters used to investigate the sows and gilts were: clinical examination (body temperature, general health and local site reactions); blood sampling; follow up by the farmer; weight (piglets); and necropsy (for any deaths during the study). A scoring system was used, and statistics applied to the main criteria (rectal temperature; systemic reactions; number of piglets born alive per gilt/sow; weaning-conception interval per gilt/sow) and to the secondary criteria (local site reactions; average daily weight gain for piglets during the suckling period; number of weaned piglets per gilt/sow).

Following injection, a significant higher temperature was recorded in vaccinated animals but the temperature increase was transient and no significant differences were observed between the treatment and control groups after 24 hours.

The percentages of gilts showing systemic reactions after 1st, 2nd and 3rd injection were, respectively:
- 8.5 % in the treatment group compared to 1.2% in the control group. The difference is not significant. In both groups, all the reactions recorded were appetite decreases 4 hours post-injection;
- 17.3% in the treatment group compared to 1.2% in the control group. The difference is significant. In the treatment group, the reactions recorded were: appetite decreases; moderate depression; lethargy. In the group B, the reactions recorded were an appetite decrease;
- 15.2% in the treatment group compared to 0.0% in the control group. The difference is significant. In the treatment group, the reactions recorded were: appetite decrease; moderate depression.

The percentages of sows showing systemic reactions after injection were:
6.7% in the treatment group compared to 1.7% in the control group. The difference is not significant. Although appetite decreases and moderate depression were observed in both the treatment and control groups, the incidence was higher in the vaccinated animals.

Systemic reactions in gilts and sows were, therefore, concluded to be moderate but transient.

The percentage of sows showing a local site reaction was 38.3% in the treatment group and 1.7% in the control group. The difference is significant. In the treatment group, the reactions recorded were redness (78% of animals) and swelling (22% of animals). In the control group only redness was recorded. Local reactions disappeared in all animals after 2 days on an average (limits not indicated).

The mean number of days (adjusted means) between the weaning and the fertile mating for gilts/sows was not statistically different between the treatment and control groups. In both cases it was reasoned that there was no farm effect and no significant interaction group/farm.

The average daily weight gain (ADG) of suckling piglets and the mean number of weaned piglet (MNWP) per gilt/sow were calculated for each group and compared to see the effect of treatment. The differences between the groups was not significant.

A compilation of the mean results obtained by taking into account other parameters presented (only as an indicator and not planned to be statistically analysed) is reported as follows:

Parameter	**Gilts** Treatment group / Control group	**Sows** Treatment group / Control group
Stillborn piglets	0.7/0.9 (median = 0)	1.5/1.2 (median value not given)
Mummified piglets	0.1/0.2 (median = 0)	0.2/0.4 (median value not given)
Suckling period mortality (%)	18.8/19.8	13.7/15.6

For the main farms, the mean antibody titres were higher in the control group than in the treatment group prior to vaccination. After vaccination, the mean values were higher in the treatment group. For all farms, the mean antibody titres of piglet sera by group sampled at weaning were higher in the treatment group than in the control group.

Field study 2:

The objective of this field study was to confirm under field conditions (infected herds, no history of previous vaccination against PCV2) in France, the safety of two i.m. administrations of Circovac vaccine. The vaccine batch used was the 1 vial (ready-to-use) presentation, however, this was fully characterised.

Three farrow-to-finish farms located in the major pig production area of Brittany in France, and identified as suffering from PMWS through lesion observations at necropsy and laboratory confirmation were included in study. Within each selected farm, four successive batches of pregnant sows were enrolled in the study, two of them being vaccinated and the other two receiving placebo (random selection).

Among the 2 vaccinated batches of sows, one was injected with a 2-ml dose of the vaccine at 12 weeks and then at 9 weeks before the scheduled date of farrowing, the other batch of sows was injected with a 2-ml dose of the vaccine at 6 weeks and then at 3 weeks before the scheduled date of farrowing.. Control animals were treated with a placebo consisting of a sterile saline solution according to the same schedule as the corresponding vaccinated group.

The safety of the vaccine was evaluated through monitoring of general and local reactions, and evaluation of the reproductive performances of the sows in each treatment group. In addition, seroconversion to PCV2 after vaccination was also evaluated.

Short term systemic and local reactions after each of the two injections were monitored on up to 20 sows per batch and on all sows if batches contained less than 20 sows. Systemic reactions examined were: rectal temperature, appetite (score system, normal, decreased, anorexia), behaviour (score system from normal to death of animals), clinical observation. Local reactions examined were: reactions at injection sites (score system applied for size/type). Reproductive parameters monitored were abortion, and the number of piglets born alive, stillborn, mummified, and weaned per sow.

Although systemic and local observation of pigs was limited (only up 4 days after vaccination), the data obtained were detailed.

In the vaccinated group, a moderate increase in rectal temperature (between +0.13°C and +0.55°C) was observed up to 2 days after the first vaccination. Temperatures were statistically significantly higher than those recorded in the control group. After the second vaccination, a moderate increase in temperature (between +0.13°C and +0.32°C) was observed up to 1 day after vaccination in treated animals.

In the control group, no systemic reactions were observed after the first injection. 9.6% of vaccinated animals showed systemic reactions which started between 4 hours and two days following the first vaccination. The incidence rates were significantly different between the treated and control groups. The reactions were moderate (decreased appetite, depression, and rarely anorexia) but nevertheless, significantly different as compared to placebo group. After the second vaccination, while no systemic reaction was recorded in the placebo group, 2.6% of vaccinated pigs showed identical systemic reactions to those observed after the first injection (that is, decreased appetite, depression, and rarely anorexia) lasting up to four days post-vaccination. 0.9% mortality was recorded amongst vaccinated animals. In all cases death was attributed to dystocia or concomitant bacterial infections causing pyelonephritis and/or septicaemia. Abortions were reported in 0.5% of vaccinated animals (between 2

and 7 weeks post-vaccination) and 0.25% of control animals (10 days post-injection). No link with vaccination was established.

Globally, after the first injection, 0.8% and 50% respectively of control and vaccinated sows showed local reactions. After the second injection, 0% and 54.9% respectively of control and vaccinated sows showed local reactions. Differences between the two groups were statistically significant in both cases. Local reactions consisted mainly of redness of limited size.

No statistically significant differences between the two groups were observed with reference to the characteristics of litters.

In summary, this field trial showed that vaccination with Circovac, as instructed in the SPC, produced moderate and transient systemic reactions, and moderate and transient increases in temperature. Local reactions were observed in approximately 50% of the vaccinates following injection of the vaccine, and in most cases these were moderate and decreased by day 3 to 4 post injection. No consequence was recorded on reproductive data, although in very limited circumstances, abortion was observed (however abortions occurred in both the vaccinated and the control groups).

Conclusion of field safety studies

The field safety studies give further information about the incidence of local reactions after the standard vaccination schedule in both gilts and sows. This has been taken into account when agreeing the wording for local reactions in the SPC.

The field trials also give information on the reproductive safety of a single dose, or a repeated single dose, of the vaccine on a much larger scale compared to laboratory studies. It is noted that the results support the vaccine's lack of effect on either the reproductive ability of sows or gilts, or on the number or condition of piglets born to them.

Environmental safety

A satisfactory Phase I assessment of risk for this inactivated vaccine, in accordance with EMEA/CVMP/074/95, is available. The final product contains no components which may exert a toxic effect and there are no pharmacologically active components included in this vaccine. On the basis of the phase I assessment, a phase II assessment is not required. Circovac is judged to present no risk to the environment.

B. RESIDUE ASSESSMENT

Residue studies have not been presented. However, the excipients and adjuvants used in the product are listed in Annex II of Council Regulation (EEC) 2377/90 and a withdrawal period of zero days was therefore accepted by the CVMP.

MRL

The following substances contained in the final product are included in Annex II of Council Regulation (EEC) No 2377/90:

Substance	MRL status	Comments
Light liquid paraffin	Included in Annex II for all food producing species	Mineral hydrocarbons, low to high viscosity including microcrystalline waxes, approximately C10-C60; aliphatic, branched aliphatic and alicyclic compounds. CR No 2804/95
Thiomersal	Included in Annex II for all food producing species	For use only as preservatives in multidose vaccines at a concentration not exceeding 0.02 %. Cr No 749/97
Adjuvant		
Sorbitan oleate (sorbitan monoleate)	Included in Annex II for all food producing species	Approved food additive (E 494) CR No 2034
Polysorbate 85 (Polyoxyethylene sorbitan trioleate)	Included in Annex II for all food producing species	CR No 1231/06

Withdrawal period

Zero days.

OVERALL CONCLUSIONS ON SAFETY AND RESIDUES

The safety of the proposed vaccination scheme was generally well supported.

The safety of Circovac was tested in three laboratory trials and two field trials with additional information from widespread use in the field in France and Germany. Safety was demonstrated to generally be in compliance with current requirements. Laboratory safety data was presented for both presentations and was deemed to be satisfactory to support the final formulation used (two vials).

Safety of one dose, of an overdose and of repeated administration of a dose/(over)dose were investigated in the laboratory in the worst case scenario, e.g., in SPF pregnant animals. The reproductive performance of these gilts was also examined.

Additional data on the basic safety of the vaccine (safety of one overdose and repeated administration of a dose) were also obtained in non-pregnant SPF gilts and data of repeated overdose in pregnant gilts were also submitted.

In all the trials, both vaccinated animals and their progeny (where appropriate), underwent thorough clinical monitoring. Individual body temperature, general body conditions, local reaction monitoring, lesion scoring at slaughter, with specific recording of the lesions observed at injection sites and on the local lymph nodes, completed by histological examination were recorded in vaccinated animals.

The SPC wording reflects the conclusions regarding temperature increases arising from use of the product.

The safety profile of Circovac is further supported by the PSUR issued for the period 1 September 2004 – 31 July 2006.

For any of the inactivated viral vaccines, the European Pharmacopoeia (Ph.Eur.) allows an increase of body temperature no higher than 1.5°C. The limited observed events of increases in rectal temperature of up to 2.5°C, and the potential negative impact of this on the general reactions and reproductive performance, have been detailed and considered. Such increases are not abnormal for oily adjuvanted vaccines. The CVMP concluded that the SPC and other product information provide a global

evaluation of the safety aspects of the vaccine and adequately reflects the possibility of any potential ADRs which could also be associated with an increase of rectal temperature.

Regarding histological lesions, although the incidence of these was high, it was considered that a veterinarian would have these drawn to their attention by the appropriately detailed warnings in the SPC and package leaflet.

It was agreed there was no clear cause-effect relationship in some isolated abortion cases reported in the safety studies. The general condition of the sows at the time of abortion was normal, and the sows fully recovered, therefore it was concluded that stress induced by vaccination could have triggered the abortions. Therefore, the use of the word of "exceptionally" in the product information was agreed.

In total 2% of vaccinated animals died in the safety studies. Of this 2%, 0.3% were animals that were euthanased for ethical reasons and 1.7% were "natural". In all cases no clear link with vaccination was found. The Committee agreed that the potential risks associated with vaccination of the product are adequately covered in the SPC and other product information and that no specific reference to death was required.

Warnings concerning local reactions, the increase of rectal temperature and the risk of abortion in pregnant animals were therefore included in the SPC as follows:

- Slight and transient local reactions normally occur after the administration of one dose of vaccine, mainly swelling (up to 2 cm^2 in average) and redness (up to 3 cm^2 in average), and in some cases oedema (up to 17 cm^2 in average). These reactions resolve spontaneously in maximum 4 days in average without any consequence on the health and the zootechnical performances.

- In clinical studies, the post-mortem examination of the injection sites performed at most 50 days after the vaccination revealed limited lesions such as a discolouration and a granuloma in the majority of animals, as well as necrosis or fibrosis in approximately half of the animals.

- Within the 2 days following the injection, an average increase in rectal temperature of up to 1.4°C can occur. Rarely, an increase in rectal temperature of higher than 2.5°C, lasting less than 24 hours, may occur.

- In rare cases, slight apathy or reduction in appetite may be observed, which should resolve spontaneously.

- Vaccination may exceptionally cause hypersensitivity reactions. In such cases, an appropriate symptomatic treatment should be provided.

- Exceptionally abortion may occur after vaccination.

In general, the safety studies presented (3 laboratory studies and 2 field trials) are well described. All safety studies were carried out in the target species, the pig. All laboratory studies were GLP compliant. The field trials were conducted in accordance with GCP. No residue studies were presented but a withdrawal period of zero days has been justified.

In terms of user safety, it is noted that the reconstitution process required in the use of the two pack presentation may increase the risk to operators of accidental self injection compared to the single/ready-to-use vial presentation. Since this is covered by the relevant descriptions in the product information and given the technical need for a two vial presentation, this is acceptable.

In conclusion, the safety of Circovac was adequately demonstrated given its current indication.

4. EFFICACY ASSESSMENT

The efficacy of Circovac was demonstrated in four laboratory trials and one field trial, some of which were performed with batches of the original ready-to-use/one vial presentation of the vaccine, however, a laboratory study demonstrated the efficacy in a vaccination/challenge model using the two vials presentation. Additional supportive field data were provided.

The laboratory trials include:

- a dose-titration study based on the serological response induced in SPF piglets which were vaccinated twice, three weeks apart, and by the intramuscular route, by several preparations of the vaccine. This study, apart from defining the minimal and maximal antigenic content of a vaccine dose, has also been considered as supportive of the demonstration of the bioequivalence between the one and two vials presentations of Circovac.
- Three challenge experiments aimed at demonstrating the basic criteria of the efficacy of Circovac. One study was intended to demonstrate the efficacy of the recommended scheme of vaccination in SPF gilts by providing the transfer of an adequate level of antibodies to their progeny followed by a challenge at 3 weeks of age with infectious PCV2. A second study was aimed at demonstrating that one injection of Circovac in conventional sows at the end of pregnancy is capable to support the claim of the vaccine following an experimental challenge with PCV2 performed on their progeny at 4 weeks of age. The third study was the primary evidence for the efficacy of the two vials presentation.

The efficacy of vaccination of sows to protect piglets against PCV2 challenge was in general assessed by the specific PCV2 titre in pigs and piglets, absence of development of clinical signs of PMWS in piglets, the characterisation of the level of PCV2 antigen in mesenteric lymph nodes, and the quantitative measurement of PCV2 DNA in the serum and faeces. Clinical signs of PMWS and the incidence of PMWS was compared in piglets from vaccinated or non-vaccinated sows. This comparison included analysis of daily weight gain, calculation of growth score, daily clinical scores including analysis of rectal temperatures and comparison of lesion scores at necroscopy. Taken all together, the results obtained from the four laboratory trials demonstrated the efficacy of the vaccine in passively immunised piglets, especially the reduction of lesions in lymphoid tissue associated with PCV2.

A GCP trial was carried out to support the results of the laboratory study and to demonstrate the efficacy of vaccination in the field. The field trial was carried out in three conventional farrow-to-finish farms, all of them located in France. Herd selection was based on the demonstration of the circulation of PCV2 in breeders and laboratory confirmation in fatteners. Clinical follow up of the offspring (in terms of incidence of PMWS/PCV2 associated disease from weaning to slaughter) of all the dams involved in the trial was the main efficacy parameter taken into account for assessing the efficacy of vaccination. Many sows and gilts (with a proportion of approximately 70% vaccinated and 30% control animals) were included in this large clinical trial which took place over a period of two reproduction cycles and was focused on the observation, throughout their life, of the offspring of all dams included in both cycles. Statistical analysis was performed on the piglets observed from weaning to slaughter. Serological follow up was conducted on a large proportion of the sows, gilts and piglets and this showed a significant increase in antibody levels in vaccinated dams. A good correlation was observed between antibody levels in vaccinated dams and maternally derived antibodies in their piglets. The overall results obtained during the two reproduction cycles indicated a significant reduction of the PCV2-associated mortality in piglets born from vaccinated sows, even though the incidence of clinical cases of PMWS decreased between the first and second cycles in both groups.

General requirements

In the absence of a specific Ph.Eur. monograph for vaccines against PCV or PMWS, the requirements for demonstrating the efficacy of the vaccine are specified in the “Requirements for immunological veterinary medicinal products” (Title II, part 7 of the annex to Directive 2001/82/EC), Ph.Eur. monograph 0062 “Vaccines for veterinary use”.

The composition of batches used in the clinical trials is summarised. Protocols of all batches are provided.

For laboratory efficacy studies, apart from the dose/response study (when a range was tested), batches of minimum potency were used. The field efficacy study was also performed with a batch of minimum potency.

LABORATORY STUDIES:

Serological response of SPF pigs to several doses and presentations of a PCV2 inactivated vaccine

The aim of this trial was to assess the equivalence of the ready-to-use/1 vial vaccine and the 2 vial presentation submitted for registration and to determine a possible dose-effect relationship for each presentation. Serological responses were determined in 3 month old SPF piglets vaccinated with different doses of the two different vaccine preparations. Blood samples were collected from each animal, prior to, and post- vaccination, for titration of ORF2 PCV2 antibodies by ELISA. The serological responses of vaccinated piglets were investigated and compared to the control unvaccinated group.

In all vaccinated groups, a serological response was observed after each injection of vaccine. When used at a dose of 2.1 or 2.4 EU, the one vial presentation and the two vials presentation induced equivalent mean titres following primo-vaccination. The same observation was made following booster vaccination with the two presentations at a dose of 2.1 EU (effect of the presentation of the vaccine). The monitoring of the serological response of the vaccinated pigs demonstrated that the two presentations of the vaccine were equivalent in terms of immunogenicity.

On the basis of these results, the minimal and standard doses were set at 2.1 and 2.4 EU, respectively. In addition, with the demonstration of equivalence in terms of induced serological response, the equivalence of the two presentations was also demonstrated.

Efficacy of the complete vaccination scheme in gilts to protect their progeny against a PCV2 challenge

This study is the first of two pivotal studies to investigate the efficacy of the complete vaccination scheme, and was a comprehensive one involving gilts. Piglets born to vaccinated gilts were compared to piglets born to non-vaccinated gilts. Clinical observations included body weight measurement, lesion and lymph nodes scores and antibody levels against PCV2, which were considered relevant for supporting the indication for the use of the vaccine against PCV2 infection.

Healthy SPF (PCV2 seronegative) gilts, approximately 10-11.5 months old at insemination, were used for this study and randomly allocated, on the basis of their body conditions, into non-vaccinated or vaccinated groups. Vaccination was performed with the 1 vial (ready-to-use) presentation. The recommended vaccination scheme was followed. Control gilts did not receive any vaccination (or placebo). Piglets were retained from the pregnant gilts (both the vaccinated and control groups) and weaned at 17 days of age. At approximately 21 days of age (four days after weaning), these piglets were challenged by intranasal inoculation of 5 ml per nostril of a viral suspension titrating 5.58 CCID50 $\log_{10}$/ml. The challenge strain was isolated in 1998 from a pig displaying PMWS in an affected herd in Brittany (France).

Blood samples were collected from all gilts prior to the first and second vaccinations, three weeks following the second vaccination, on the day prior to third vaccination, three weeks after the third vaccination (which corresponded to the week of farrowing) and at weaning. Blood samples were collected from piglets during the first week of life, at weaning, on the day of PCV2 challenge and at days 14 & 28 post challenge. Faecal samples were taken from piglets on the day of challenge and then twice weekly until slaughtering. Daily clinical observations, including rectal temperatures, were

performed on the piglets including observations of prostration, anaemia, vomiting, dyspnoea, cough, anorexia, and any other sign recorded during the observation period. Body weight and growth scores were measured in all piglets on the day of challenge, and then weekly until slaughtering. All piglets underwent an exhaustive necropsy examination (on day 29-30), in particular of mediastinal and mesenteric lymph nodes, and according to a pre-definite scoring system. Analytical testing included serology of anti-PCV2 antibody levels, characterization of PCV2 in faeces (by qualitative and quantitative PCR), in serum (by quantitative PCR) and lymph nodes (by immunochemistry), although PCR was considered for information purposes (due to a few false positive pre-challenge results) and immunochemistry was not in compliance with GLP standards. A complete analysis of the results obtained from this challenge model was provided.

The trial evaluated the efficacy of vaccination of gilts to protect their piglets against PCV2 challenge by assessment of the specific PCV2 titre in dams and piglets, development of clinical signs of PMWS in piglets, the characterisation of PCV2 protein load in mesenteric lymph nodes, and quantitative measurement of PCV2 DNA in the serum and faeces. Evaluation of clinical signs of PMWS included analysis of daily weight gain, calculation of growth score, daily clinical scores with analysis of rectal temperatures and comparison of lesion scores at necroscopy.

For all organs observed, lesions were observed more frequently at necroscopy in piglets born to control gilts compared to piglets born to vaccinated gilts, especially in the mesenteric lymph nodes. The pattern of PCV2 ORF 2 antigens in the mesenteric lymph nodes was visualised by immunohistochemistry and this showed that there was a clear difference in piglets from vaccinated gilts compared to piglets from control gilts. For example, 63% of piglets from vaccinated gilts were negative for PVC2 antigen whereas only 18% piglets from non-vaccinated dams were negative.

The recovery of PCV2 virus in serum was significantly less in piglets born from vaccinated gilts.

During the follow-up of this study, the average level of PCV2 DNA excreted in the faeces by piglets from vaccinated sows was found to be significantly lower than that by piglets from non-vaccinated sows.

An overall global growth score was assigned, taking into account growth during the pre-vaccination period, pre-challenge period and post-challenge period. Challenged piglets from vaccinated gilts and unchallenged piglets from non-vaccinated gilts had better growth patterns than challenged piglets from unvaccinated gilts.

The antibody titres in piglets born from vaccinated gilts were consistent with the titres in the blood of their dams and remained so until challenge (3.48 $\log_{10}$ at 3-4 weeks of age compared to <0.81 in piglets born from non-vaccinated gilts). There was a relationship between the titre in the gilt after farrowing and the level in the piglet in the first week of life.

Efficacy of the complete vaccination scheme in sows to protect their progeny against a PCV2 challenge

The second of the two pivotal laboratory studies was aimed at demonstrating the efficacy of one dose of vaccine administered at the end of pregnancy to conventional sows in order to protect their progeny against a PCV2 challenge. It was a comprehensive study of vaccination of sows (originating from a commercial herd contaminated with PCV2 selected from field trials) and the subsequent challenge of piglets born to those dams (in comparison with piglets born to non-vaccinated sows). Efficacy was investigated by looking at the positive impact of vaccination of sows in relation to parameters such as clinical score, body weight, lesion score, virus excretion and PCV2 levels in pigs born to the vaccinated sows and submitted to a virulent PCV2 challenge after weaning.

SPF piglets seronegative for PCV2 acted as additional control animals.

The sows were in their first to fifth pregnancy when selected for the study. Sows selected for being vaccinated were injected 21 days before farrowing with a single dose of 2 ml of the vaccine under test. Non-vaccinated sows did not receive any treatment. Challenge was performed in the piglets at approximately 4 weeks of age. The characteristics of the challenge virus as well as those of experimental conditions (clinical observations, body weight measurements, lesion and lymph nodes scores, sample collection during the study, e.g. blood and faeces, as well as at necropsy, e.g. mesenteric and mediastinal lymph nodes) were similar to those applied in the previous challenge trial. Major data were gathered from daily observations of animal health status carried out starting the day before and ending 28 days after challenge. Mortality and rectal temperature were recorded, and the piglets' general health (anaemia, vomiting, anorexia, etc) monitored. Individual weightings were performed on each piglet at challenge (D0), and then weekly (e.g. D7, D14, D21 and D28) until slaughter (at D29). A necropsy examination was carried out on the same day on all piglets (using the same experimental conditions adopted in the previous challenge experiment). Analytical testing also included serology of anti-PCV2 antibody levels. In addition to such testing, anti-PRRS antibody and characterization of PRRS in the piglets' sera by RT-PCR were also tested in order to ascertain the potential suspected involvement of PRRS in the intercurrent pathology observed in piglets. PCV2 in faeces was characterised by PCR and in the mediastinal lymph nodes by immunochemistry. Adverse events and concurrent treatments were recorded. In particular, it was mentioned that an episode of respiratory disease apparently associated with *Actinobacillosis* and *Streptococcal* septicaemia occurred in piglets born from vaccinated sows after challenge in parallel with an outbreak in the farm of origin.

The challenge model was validated on the basis of the high mean clinical scores and antibody titres recorded in piglets in the control group (seronegative to PCV2/no virus detected in faeces before challenge).

Antibody titres were similar in sows of both test groups before vaccination. The antibody titres in vaccinated sows after farrowing were higher and less variable than the titres of non-vaccinated sows. The antibody titres of piglets born from vaccinated sows were higher and less variable than the titres from piglets born from non-vaccinated sows.

The piglets born to vaccinated and non-vaccinated sows were challenged at approximately 4 weeks of age.

The two test groups exhibited a similar growth pattern during the follow-up of this study.

Piglets born from vaccinated sows exhibited fewer lesions after challenge. No statistically significant difference was recorded int eh frequency of lesions recorded in mediastinal lymph nodes; by contrast, no lesions were observed in the mesenteric lymph nodes of pigs born from vaccinated sows, whereas >50% of piglets from the other two groups presented lesions scoring $\geq$2 (67% and 82% respectively).

A high number of SPF control piglets developed lesions in the lymph nodes after challenge and 50% had more than 1 focus/field in immunochemical detection of PCV2 ORF2 antigens in the lymph nodes.

This study investigated vaccination in a PCV2 infected herd and used only one injection 2 weeks before farrowing which could be taken as a worst case scenario.

Efficacy of Circovac in SPF pregnant gilts for passive protection of their offspring against a PCV2 challenge, and duration of immunity

The aim of this detailed and well presented study was to demonstrate the efficacy of the vaccine, based on serology in the dams and in the piglets and on protection against a virulent PCV2 challenge (intranasal route) in the piglets up to 4-5 weeks after passive transfer of antibodies, and to demonstrate the duration of immunity (DOI).

SPF, 10-11 month old at insemination, pregnant gilts were vaccinated in accordance with the proposed vaccination scheme. In contrast to some of the earlier studies, the final formulation (2 vial presentation) was used. The control group comprised SPF pregnant gilts which did not receive a vaccination (no placebo).

Serology of the gilts and their piglets was investigated. In the piglets follow up lasted for 28 days after challenge with PVC2 intranasally (as before) when the piglets were 4-5 weeks of age.

The parameters checked in the piglets in order to assess the protection against challenge were: examination of body weight, clinical signs, including prostration, dyspnoea, anaemia (skin colour) cough, anorexia, vomiting and measurements of rectal temperature (based on a scoring system), lesions at necropsy, including examination of external appearance, thoracic and abdominal cavities (based on a scoring system), viral load and histological lesions in lymph nodes.

Apart from transient episodes of vomiting in 19% of gilts, the vaccine was well tolerated by the vaccinated pigs.

Body weight of the piglets: after challenge, the evolution of body weight was similar in all piglets (either born from vaccinated or control dams).

Although statistical analysis confirmed that the difference between clinical signs observed in vaccinated and control piglets was significant, the challenge did not induce PMWS cases. Rectal temperature comparisons showed a statistically significant difference between the piglets born from vaccinated dams and piglets born from control dams 14 days after challenge Lesions at necropsy, virus load in mesenteric lymph nodes and histology supported the outcome of the challenge.

The results of the serological profiles from this study demonstrated a specific and strong seroconversion in the vaccinated gilts (seronegative at the beginning of the trial) and the efficient transmission and persistence of antibodies in their piglets following colostrum intake.

The claim for reduction in lesions (caused by PCV2 challenge) was supported.

The impact of vaccination is evident on lesions induced by the challenge virus in the regional lymph nodes. A 50% reduction of virus load in mesenteric lymph nodes can be acknowledged. A positive trend only was demonstrated for the protection against clinical signs, although the challenge induced only moderate clinical signs of PMWS. The results were considered supportive for the claim for protection against histological lesions in mesenteric lymph nodes in piglets up to 4 to 5 weeks after the transfer of passive immunity via the colostrum intake from vaccinated dams.

FIELD TRIALS

Efficacy of vaccination in a field trial carried out in three conventional farrow-to-finish farms

The same vaccine batch used in the two laboratory challenge experiments was used to confirm the efficacy of vaccination in a field trial carried out in three conventional farrow-to-finish farms, all of which were located in France.

Prior the study, suspected PMWS in piglets was confirmed by necropsy during the inclusion visit, and positive serology in reproducers confirmed the circulation of PCV2 in the herd. Clinical follow up of the offspring (in terms of incidence of PMWS from weaning to slaughter) of all the dams involved in the trial was the main efficacy parameter. Approximately 70% of the sows and gilts were vaccinated whilst the remaining 30% formed the unvaccinated control group.

This large trial took place over a period of two reproduction cycles and was focused on the observation, throughout their life, of the offspring of all dams included in both cycles. Serological

follow up was conducted on large numbers of the sows, gilts and piglets. Statistical analysis was performed on the piglets observed during their entire life from weaning to slaughter.

There was no overall significant difference between the piglet groups and their mortality; however, during the second reproduction cycle a significant difference was detected in that the piglets coming from vaccinated sows exhibited a lower mortality rate.

From the results it was concluded that the incidence of clinical cases of PMWS, as calculated by the sum of piglets with appropriate clinical scores, significantly decreased between the first and second cycles in both the vaccinated and control groups. The overall percentage during the first cycle was respectively 3.2% and 1.7% in the control and vaccinated groups. During the second cycle the overall percentage decreased to 1.2% and 0.4% respectively in piglets born from non-vaccinated or vaccinated animals. The overall % values of clinical suspicion/confirmation of PMWS over the two cycles were 2.15 in control animals and 1.02 in test animals.

As far as the incidence of PMWS mortality is concerned, the results showed double the reduction in PCV2 linked mortality in vaccinated animals compared to control animals. A good correlation between the antibody levels in dams and maternally derived antibody in piglets, in both the vaccinated and control groups, was revealed.

Additional efficacy information

Additional information from the use of the product in Germany and France was submitted to support the claims for Circovac aiding in the reduction of mortality of the piglets.

Piglets born to vaccinated sows in 63 farms in France and Germany showed between a 3.6% and 10% reduction of piglet mortality under field conditions. In those two countries, a great variability was initially present in the 63 farms studied. These diverse characteristics and the geographical distributions of the farms reflected some of the current diversity of swine production whilst being very typical of national and European practices. Despite this initial variability, after vaccination of the breeder herd, almost all the farms experienced a benefit in piglet mortality rates up to time of slaughter: almost 100% of French farms, and 60% to 90% of the German farms, depending on the age group, exhibited a substantial improvement with a corresponding decrease of mortality in piglets born from vaccinated females. Despite this initial variability, results were consistent in the 22+3 French farms and the 38 German farms. The improvement was associated with farms with mortality higher than the acceptable level, with a correlation between mortality rates before vaccination and the importance of improvement. The higher the mortality before vaccination, the more likely an improvement was observed and the more intense the improvement.

These results showed that the vaccine performed well in quite diverse epidemiological situations.

OVERALL CONCLUSION ON EFFICACY

From the results of the efficacy studies performed, it may be concluded that Circovac is efficacious at 2.1 EU/ml per (2 ml) dose and that reduction in lymphoid lesions is obtained. The duration of immunity of Circovac in piglets from vaccinated sows and gilts is up to 5 weeks after transfer of colostrum.

The claim agreed is “Passive immunisation of piglets via the colostrum, after active immunisation of sows and gilts, to reduce lesions in lymphoid tissues associated with PCV2 infection and as an aid to reduce PCV2-linked mortality.”

In conclusion, the efficacy of Circovac was adequately demonstrated given its current indication.

5. BENEFIT RISK ASSESSMENT

The analytical part of the dossier is correctly documented, especially with regard to the production and control of the PCV2 antigen component and control of all the starting materials. The data provided demonstrate adequate control of all the manufacturing processes, which leads to the production of a finished product of defined and consistent quality.

Shelf-lives for both the finished product (as marketed) and the reconstituted product at the proposed storage temperatures have been justified.

The TSE risk for this product can be regarded as negligible.

The safety of the proposed vaccination scheme was generally well supported.

The safety of Circovac was tested in three laboratory trials and two field trials with additional information from use in the field in France and Germany. Safety was demonstrated to be in compliance with current requirements. Laboratory safety data were presented for both presentations and was deemed to be satisfactory to support the final formulation used (two vials).

Safety of one dose, of an overdose and of repeated administration of a dose/(over)dose were investigated in the laboratory in the worst case scenario, e.g., in SPF pregnant animals. The reproductive performances of these gilts and sows were also examined.

Additional data on the basic safety of the vaccine (safety of one overdose and repeated administration of a dose) were also obtained in non-pregnant SPF gilts and data from repeated overdoses in pregnant gilts were also submitted.

In the absence of specific information regarding potential for interference with vaccine uptake posed by the concurrent use of other vaccines commonly administered late in gestation (in particular those which may cause additional temperature increase, thus increasing the risk of abortions), it was considered prudent to rely on further information on ADRs made available from the ongoing use of the vaccine in the field (namely in France and Germany). The SPC wording reflects the conclusions regarding temperature increases arising from use of the product.

It was agreed there was no cause effect relationship in the 2 abortion cases reported in the safety studies. However, serious SARs related to the use of Circovac were reported (in February 2007) during a clinical trial in Germany. The general condition of the sows at the time of abortion was normal, and the sows fully recovered, therefore it was concluded that stress induced by vaccination could have triggered the abortions. Therefore, the use of the word of “exceptionally” in the product information was agreed.

Warnings concerning local reactions, the increase of rectal temperature and the risk of abortion in pregnant animals were, therefore, included in the SPC.

A withdrawal period of zero days has been justified.

User safety of the product is considered acceptable.

Circovac is judged to present no risk to the environment.

With regards to efficacy, a reduction in histopathological lesions in lymphoid tissues, associated with PCV-2 infection, was demonstrated in the critical laboratory studies.

From the results of the efficacy studies performed, it may be concluded that Circovac is efficacious at 2.1 EU/ml per (2 ml) dose and that reduction in lymphoid lesions is obtained. The duration of immunity of Circovac, demonstrated in laboratory studies, in piglets from vaccinated sows and gilts is up to 5 weeks after the transfer of colostrum.

In the field, in piglets born from vaccinated herds, a reduction of mortality between 3.6% and 10% over the pig(let)s' economical life was noted.

In conclusion, the efficacy of Circovac was adequately demonstrated given its current indication.

In reviewing the overall data related to safety and efficacy of this vaccine, the Committee noted:

a) that globally the general safety profile is expected to be similar to other oily adjuvanted vaccines;

b) the rationale for building the vaccine strategy.

In the following table, the risks and benefits associated with the use of this vaccine are summarised:

Risks	Benefits
- Up to a 1.4°C increase in body temperature, and rarely >2°C, and slight apathy or reduction in appetite for about 48 hours post-vaccination in the gilt/sow - Transient induration, redness, and in some cases oedema in the gilt/sow - Discolouration, granulomatous inflammation, and some muscle lesions at the injection sites in the gilt/sow - Exceptional hypersensitivity reactions in the gilt/sow - Exceptional abortion cases - Risk of injection of oil adjuvant to humans	- Reduction of lesions in lymphoid tissues associated to PCV2-infection in the piglets - Between 3.6% and 10% reduction of mortality over the economical life of pig(let)s born from vaccinated herds - Improvement of the health status in the farm - Improvement of food conversion ratio in the piglets - Decrease in the use of medication especially antibiotics in the herd

Based on the original and complementary data presented the Committee for Medicinal Products for Veterinary Use concluded that the quality, safety and efficacy of the product was considered to be in accordance with Directive 2001/82/EC as amended.