SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

Ingelvac CircoFLEX is a biotechnology derived inactivated immunological veterinary medicinal product. This vaccine is intended to be used for the active immunisation of pigs against acute and chronic forms of Porcine Circovirus Disease (PCVD). First recognised in 1991 in Canada, Porcine Circovirus Type 2 (PCV2) is considered to be associated with a number of disease syndromes which have been collectively named PCVD. In particular, infection by PCV2 is nowadays recognised as the major risk factor in the establishment of the complex syndrome known as Post-weaning Multi-systemic Wasting Syndrome (PMWS) which affects principally piglets at the post-weaning and beginning of fattening stages. Ingelvac CircoFLEX administered once via the intramuscular route to pigs from two weeks of age is capable of reducing mortality, clinical signs (including weight loss) and lesions of acute and chronic PCVD in addition to a reduction of PCV2 excretion, viral load and virus persistence in the body.

The vaccine antigen is the major capsid protein encoded by a specific gene (the Open Reading Frame 2, ORF2, gene) of Porcine Circovirus Type 2. This antigen is recognised to be highly immunogenic and it is produced via an insect cell Baculovirus Expression System (BEVS). The baculovirus has been modified by introducing the ORF2 gene encoding PCV2 ORF2 capsid protein. ORF2 antigen is secreted in the antigen production medium. This reaction is stopped by inactivation of the Baculovirus Expression System.

To ensure that a batch of vaccine will lead to the claimed efficacy, its relative potency (RP) is determined. An ELISA potency test was developed in order to establish a correlation between the RP of the vaccine under test and the RP of a reference preparation which gave optimal results in an efficacy study. No preservative is added as the product is recommended for immediate use.

2. QUALITY ASSESSMENT

Composition

Ingelvac CircoFLEX is a suspension for injection with each dose of 1 ml of inactivated vaccine containing as active substance Porcine circovirus type 2 ORF2 protein, minimum relative potency 1.0, maximum relative potency 3.75 (Relative Potency ELISA test by comparison with a reference vaccine). Carbomer is used as an adjuvant and as excipient sodium chloride 0.85% solution in water for injection in bulk.

Container

The vaccine is filled in natural high density polyethylene (HPDE) 15, 60, 120 or 250 ml capacity bottles to contain respectively 10, 50, 100 and 250 doses of 1 ml. The different pack sizes allow the user to adjust the vial size to the number of animals to be vaccinated at one time. The bottles are pre-sterilised by gamma irradiation. The bottles are closed with 20mm (10-50-100 dose bottles) or 30mm (250 dose bottle) of siliconised chlorobutyl rubber stoppers. The stoppers are sterilised by steam heat using a validated autoclave cycle. Fixation of the rubber stoppers on the bottles is ensured by lacquered aluminium seals. Satisfactory certificates of analysis were provided.

Development Pharmaceutics

The product has been developed as a vaccine against PCV2 based on the baculovirus technology used to express ORF2 capsid protein of PCV2. The development of such a subunit vaccine was based on evidence that ORF2 has been scientifically recognised to be a major immunogen protein, taking advantage of particular characteristics of the baculovirus expression system. The active component of the vaccine is the PCV2 ORF2 protein which is expressed in insect cells after inoculation with a baculovirus vector containing the ORF2 gene of PCV2. ORF2 antigen will induce protection of piglets against a PCV2 challenge, based on the generation of an active immune response against the virus.

The suitability of the field isolate of PCV2 (isolated in North America from pigs suffering clinically from PCVD) that was used for production of the vaccine for all areas of the EU was justified based on published literature as well as on the results of the efficacy studies conducted in different regions of the European Union. PCV2 strains are genetically stable with respect to their immunogenic properties.

The processes of cloning and expression of the PCV2 ORF2 gene have been described in detail. Details of the genetic engineering, the cloning steps, technical specifications etc. have been provided. Critical steps in the manufacturing of the final immunological product have been investigated, including the production of vaccine antigen in bioreactors, the downstream and inactivation processes.

The addition of an adjuvant to the vaccine antigen has been used in order to maximise the immune response to the active substance in the target animal. An innovative adjuvant-vaccine combination was selected given a series of perceived advantages mainly correlated with the physical-chemical characteristics of the adjuvant. The adjuvant is a cross-linked carbomer-polymer containing sodium chloride and water for injection which has been extensively and safely used either as an emulsifying or adjuvant agent. Moreover, it produces an aqueous formulation (that has low viscosity in comparison to water-in-oil emulsions) which minimises local reactions at the injection site and allows the vaccine to be combined with other vaccines. MRLs (Maximum Residue Levels) do not need to be established because carbomer is considered as not falling within the scope of Council Regulation (EEC) No. 2377/90 (EMEA/CVMP/046/00). The choice of physiological saline (0.85% solution of sodium chloride in water for injection in bulk) as the diluent was justified.

The packaging materials were selected in accordance with specifications for a water-based biological product according to Ph. Eur. and packaging components comply with Ph. Eur. as well. The product is recommended for immediate use, and no preservative is added thus reducing the use of potentially toxic constituents.

Relevant information concerning the manufacture of batches used in clinical trials has been provided, with batches containing the maximum and minimum amount of ORF2 antigen and used respectively in safety and efficacy studies.

Method of manufacture

Manufacture is realised in two phases; production of the PCV2 ORF2 antigen lot(s) and vaccine blending. The entire production procedure is performed in the GMP (Good Manufacturing Practice) certified production facility. General and detailed flow charts have been provided of the steps taken to produce and test the quality of the vaccine. SOPs (Standard Operating Procedures) have been provided for the critical aspects of production. Steps involve SF+ cell culture, ORF2 antigen production culture, antigen inactivation and harvest, vaccine blending, bottling and packaging.

The PCV2 ORF2 manufacturing process is fully described and details have been provided for the control or monitoring of each step. All processes are carried out under closed operation conditions and all connections are steam-sterilised to ensure aseptic processing. Inactivation of live baculovirus is performed and satisfactory inactivation kinetics results were provided.

The vaccine antigen is considered appropriately pure because of the production system applied (e.g. culture medium used for ORF2 production is completely protein-free and there is only one expression product, i.e. ORF2 antigen). BEI (Binary Ethylenemine) inactivation, and inactivation control, ensures that no live baculovirus is present in the final product. Moreover, taking into account the specific mode of BEI inactivation, the inherent controls of the production process and of starting materials, the risk of other genetic material being present is nil.

The antigen content is determined after inactivation and prior to blending by an ELISA in-process test. Antigen lots may be pooled to formulate the finished product during the blending procedure. Blending of the vaccine is made according to the ELISA test results. The bulk vaccine is prepared by one or several lot(s) (containing the appropriate amount of filtered and inactivated ORF2 antigen blended with carbomer and diluent (physiological saline solution). All addition, sampling and transfer steps are carried out according to GMP under sterile closed operation conditions. The bulk is transferred to the automated fill line and filled in 15, 60, 120 and 250 ml plastic bottles for 10, 50, 100 and 250 dose presentations, respectively. The bottles are made of high density polyethylene. Bottles are pre-sterilised by irradiation by the manufacturer, the stoppers are sterilised by autoclaving. Filling operations are carried out under aseptic conditions and adequately monitored.

Validation studies

The antigen manufacturing process was validated in bioreactors. Data related to successful completion of lots of antigen at production scale have been presented to validate the vaccine manufacturing process. Details of all activities regarding the validation of the manufacturing process, including critical parameters have been reported. Antigen production batches will be produced in the exact same vessels that were used for process validation. All process parameters will be kept within the validated ranges, hence the batch data provided are representative of those proposed for routine production.

Additional data and information were provided in order to characterise vaccine antigen and to ensure consistency of total protein profiles in final vaccine batches. No additional effect on immunogenicity is expected from non-ORF2 proteins present in the final product.

Therefore, it can be concluded that the manufacturing process is robust and generates consistent vaccine batches in light of the use of an established, tested seed lot system with an established, validated and controlled manufacturing process, the use of validated in-process tests and extensive testing of finished product, identification of the protective antigen – PCV2 ORF2, qualitative characterisation of the protein profile by SDS-PAGE and use of a specific, validated ELISA to quantify the protective antigen.

CONTROL OF STARTING MATERIALS

Conventional pharmaceutical excipients are used and they comply with the relevant pharmacopoeial monograph.

Carbomer (Ph. Eur.)
Sodium hydroxide (Ph. Eur.)
Water for injection in bulk (Ph. Eur.)
Sodium thiosulphate (USP)

The materials used in the media preparation consist of those listed below and all comply with the relevant European Pharmacopoeia monograph.

Sodium chloride (Ph. Eur.)
Sodium bicarbonate (USP)
Neomycin sulphate (Ph. Eur.)
Potassium hydroxide (Ph. Eur.)
Hydrochloric acid (Ph. Eur.)
Water for injection (Ph. Eur.)

Starting materials, preparation, in-process and batch control, validation methods, function and storage conditions have been sufficiently described and supported by adequate documentation.

Starting materials of biological origin

PCV2 ORF2 recombinant baculovirus and SF+ cells

For PCV2 ORF2 recombinant baculovirus, details were provided of how the Baculovirus Expression System (BEVS) was constructed in order to contain all the signals capable of optimising correct transcription and translation of heterologous genes in cell hosts in which expression takes place. Details of suitability of plasmid transfer vectors and of testing have been provided. Details regarding the preparation and description of Master Seed Virus (MSV) and Working Seed Virus (WSV) have also been provided. Complete sets of controls according to current EU legislation have also been provided for both MSV and WSV. The genetic stability of MSV and WSV (from MSV+1 to MSV+4) was demonstrated.

An available insect cell line derived from Spodoptera frugiperda (SF) and denominated SF9, has been used as an adherent, serum dependent cell line for the construction of the recombinant baculovirus containing the ORF2 gene. For ORF2 antigen stocks production and protective protein expression, the engineered PCV2 ORF2 baculovirus is propagated on commercially available SF cells derived from SF9 cells. SF9 cells were adapted for large scale production to grow as a serum-free suspension cell line and were designated SF+. An extensive description and testing have been reported for the parental SF cell lines. Tests conducted included sterility (Ph.Eur. 2.6.1), Mycoplasma (Ph. Eur. 2.6.7), identification of species (9CFR 113.52b)/ isoenzyme analysis and karyology. There was also an examination for the absence of extraneous agents in accordance with Ph. Eur. requirements.

Vaccine production is made in subcultures of SF+ cells. Results of identity, sterility, mycoplasma and extraneous agents tests conducted in accordance with Ph. Eur. requirements were provided. Further testing of the highest working passage used for production was carried out in order to demonstrate that the production cell culture is similar to MCS with regard to biological characteristics and purity (results of testing for sterility, mycoplasma, identification of species by isoenzyme analysis, as for Ph. Eur., karyology, as for established procedure and viral contaminants).

Starting materials of non-biological origin

BEI (Binary Ethylenemine)

Quality is guaranteed by adequate information related to starting materials, or describing preparation, in-process and identity test, validation methods, function and storage conditions.

In-House preparation of media

A description of the method of preparation of the in-house media used, including the sterilisation procedure and its validation, controls and tests carried out have been sufficiently described and supported by adequate documentation. The information provided reassurances regarding the quality of this medium.

SPECIFIC MEASURES CONCERNING THE PREVENTION OF THE TRANSMISSION OF ANIMAL SPONGIFORM ENCEPHALOPATHIES

Assessment of starting materials has been conducted in compliance with Commission Directive 1999/104/EC and in accordance with the Note for Guidance on minimising the risk of transmitting agents via veterinary medicinal products (EMEA/410/01-Rev.2). None of the components of the final product is derived from any mammalian animal source and a declaration of compliance has been provided.

CONTROL TESTS DURING PRODUCTION

Various SOPs apply to the in-process sterility test and relevant information to support the validity of the tests and of the controls has been provided. An in-process mycoplasma test is carried out (according to Ph. Eur. Monograph 2.6.7) on ORF2 protein antigen prior to filtration and inactivation with the aim to monitor antigen for mycoplasmal contamination. The procedure for inactivating viral antigen fluids and for testing viral antigen fluids for complete inactivation has been described in detail. The test is carried out on the inactivated and neutralised material and again on the bulk antigen before blending according to Eur. Ph. Requirements. ORF2 quantification is by ELISA and identification assay and the antigen content is measured in each lot after inactivation and neutralisation. The same test used to quantify ORF2 is also used to confirm the identity of the active substance in each lot of harvest.

A parallel line method is used to determine potency. In order for a lot of antigen to be satisfactory, it must have an RP (Relative Potency) of ≥ 1.0 using the reference standard lot (medium titre sample RP=1). A validation study was performed to demonstrate specificity, precision, accuracy, linearity/range and robustness of the ELISA test.

CONTROL TESTS ON THE FINISHED PRODUCT

The final vaccine is an aqueous sterile inactivated vaccine. The following tests are carried out on the finished product: sterility, target animal safety, batch potency/identity, carbomer content and pH. Concerning sterility a number of SOPs apply for the in-process sterility test concerning Ph. Eur. sterility testing of biological products, microbiological media validation: growth promotion, bacteriostasis/ fungistasis testing for validation of the sterility test for final products. Sterility is tested on each batch or sub-batch of final product. Target animal safety is tested on each batch or first sub-batch of final product. Details of the test and specifications have been provided. The test batch is satisfactory if after the intramuscular injection of a double dose of the vaccine into two 3-week old-pigs, followed by a 3 week observation of the animals for the appearance of any adverse local or systemic reactions including increase of body temperature and negative influence on growth rate, the piglets do not exhibit any severe untoward reactions attributable to the vaccine. No relevant impact on normal animal performance is expected to occur. CVMP has recognised that for this specific vaccine, the use of 3 week old piglets for routine batch safety tests can be considered equivalent to the use of piglets of the minimum age for vaccination (2 weeks).

Batch potency test (BPT) and identity assay for PCV2 vaccine potency and identity test is performed in line with a specific SOP. An *in vitro* potency ELISA assay was developed and validated in order to measure PCV2 ORF2 protein expressed as RP of each batch or sub-batch of final product in respect to a reference vaccine that was demonstrated to be efficacious in the host animals. Examples of a trend analysis for monitoring the stability of the reference vaccine used as control sample in BPT were also provided.

With regard to pH and visual appearance testing a specific SOP concerns calibration, operation and maintenance of quality control pH meters and the test is performed on each batch or sub-batch of final product. A macroscopic observation of the final, bottled product is carried out where the vaccine appears as a transparent to moderate turbid, colourless to yellowish, non-viscous suspension.

Concerning batch to batch consistency a number of batches were manufactured and tested in accordance with the relevant methods outlined in the dossier. These were used to validate the relevant batch size and gave satisfactory results as far as sterility (the batches showed full compliance with Ph. Eur. requirements, resulting free of bacterial and fungal contamination) and target animal safety (no local/systemic reactions occurred) are concerned. Identity (specific reaction to PCV2 ORF2 monoclonal antibody, as for relevant SOP) was confirmed for the batches in question; visual appearance demonstrated that the vaccine can be produced as a transparent to moderately turbid, colourless to yellowish, non-viscous suspension. Consistency of production was demonstrated.

STABILITY

The stability of the master reference preparation is monitored periodically by testing retention samples in either the host animal or an equivalent *in vivo* potency assay. For the identification and assay of adjuvants carbomer content is assessed in line with a specific SOP and a validation study concerning validation of the sedimentation method for quantifying carbomer in serials containing PCV2 ORF2. This latter test is carried out on each batch or sub-batch of final product. The test has been validated, in terms of accuracy, precision, linearity and robustness. The percentage of carbomer accepted in the final product must be within a specific range. Criteria for batch rejection or for invalid results have also been defined in the test protocol.

Stability of the bulk antigen

Real-time and accelerated stability analysis (by SDS-PAGE and ELISA) was performed with satisfactory results, the objective being to provide an estimate of acceptable storage time for ORF2 bulk fluids. The results demonstrated that the bulk antigen can be safely stored at 4°C for up to 12 months.

Stability of the bulk vaccine

Real time stability testing carried out up to 18 months on batches of finished product blended from a bulk of vaccine kept for approximately 5 days at 1°C- 7°C, supported a maximum storage period for a bulk of vaccine prior to final blending of up to 5 days at 1°C- 7°C.

Stability of the finished product

Results up to 18-24 months have been provided for bracketing dose preparations (e.g. min/max dose preparations).

Test results and compliance with specifications (as for the final product testing Batch to batch consistency) at release have been provided and have been satisfactory. Based on these data a shelf life of 15 months is justified.

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 the vials of the product are well described and the in-process controls detailed in full. The compliance of starting materials 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 the product 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 15 months for the finished product is justified. The SPC and other product information include all the necessary information regarding storage and use of the product.

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

3. SAFETY ASSESSMENT AND RESIDUES

Ingelvac CircoFLEX is presented as a suspension for injection and is an inactivated subunit vaccine. It is intended for active immunisation against Porcine Circovirus type 2 (PCV2) to reduce mortality, clinical signs - including weight loss - and lesions in lymphoid tissues associated with PCV2 related diseases (PCVDs). In addition, vaccination has been shown to reduce PCV2 nasal shedding, viral load in blood and lymphoid tissues, and duration of viraemia. Onset of protection occurs as early as 2 weeks post-vaccination and lasts for at least 17 weeks. Pigs are vaccinated from 2 weeks of age by a single intramuscular injection of one dose (1 ml), irrespective of body weight. The safety of this proposed vaccination scheme was supported.

Two laboratory and three field studies were carried out in order to investigate potential risks deriving from the use of the vaccine. All laboratory trials were performed using negative-controlled, randomised and double blinded designs and were carried out under GLP (Good Laboratory Practice) conditions. All studies were conducted and monitored by qualified veterinarians or scientists and in case of *post mortem* examination the analysis was performed by an experienced pathologist.

Laboratory and field safety studies were conducted involving the administration of one dose, overdose and repeated dose. All safety studies were performed in the target species for vaccination, using the most sensitive category of susceptible animals, e.g. piglets of minimum age (2 weeks). Caesarean-derived and colostrum–deprived piglets (CD/CD), kept under isolated conditions were selected for laboratory safety studies, the choice being supported by the extreme susceptibility of this category of animals which by virtue of selected origin and due to the lack of protection by maternally derived antibodies are expected to be more susceptible to infections with bacterial and viral pathogens. CD/CD piglets are also expected to more likely respond with adverse events to vaccination than conventional piglets, thus providing an appropriate model for safety evaluation under experimental conditions.

Commercial crossbred piglets approximately 2 weeks of age were initially selected for the two laboratory studies. As part of the trial, piglets were injected with the “test article” vaccine batch and piglets representing the control group receiving a “reference article” (water for injection). The study animal population was homogeneously selected for sex and breeds. Commercial cross-bred genetic lines were included in the field studies representing sow and boar lines typical for European pig production (Duroc, Large White, Landrace, Pietrain). Selection of farms was carefully evaluated in order to cover the whole range of PCV2 seropositive animals (established to be considered as low: <1:100; moderate: <1:1000; high: >1:1000). All safety studies were conducted with a vaccine batch formulated to contain the maximum RP expected to be found in a dose of vaccine (3.75/ml when compared to the reference batch vaccine). As the selected route of administration is intramuscular, the vaccine was always administered intramuscularly in the neck muscle.

Parameters used to demonstrate safety used evaluation criteria such as local reactions (clinical examination of injection sites *in vivo* and microscopic examination of injection site *post mortem*), systemic reactions (general health, rectal body temperature and *post mortem* analysis with special emphasis on kidneys), performance parameters (average weight and average daily weight gain), immunological parameters (PCV2 antibody titre) and infectious status (PCV2 induced viraemia). The latter two parameters were also used to characterise the infectious status of the animals included in the studies.

Histology was used for detection of alterations at the injection site as well as in kidney tissues. Immunohistochemistry (IHC) was used to identify and detect PCV2 antigen in de-paraffinised tissue section slides derived from formalin-fixed porcine kidney material. The results obtained from IHC testing are valuable and well accepted for the diagnosis of PCV2 due to the very high degree of correspondence with the results obtained from other tests and the presence of macroscopic lesions after infection. IHC was validated and testing was carried out according to relevant SOPs and assay method protocols (AMPs). All pathological examinations were performed under controlled conditions and each analysis included positive and negative controls to validate the staining and analysis process.

A thorough description was provided of the validation of nucleic acid extraction and polymerase chain reaction amplification of DNA for the detection of PCV2 from cell free fluids (e.g. serum, swab and cell culture fluids), as well as in organ materials (e.g. kidney tissue or other cells containing material) were provided. Detection of anti-PCV2 antibodies in individual serum samples by IFAT (immuno-fluorescence antibody test) was performed by laboratories which had participated in a cross-validation of the method in both laboratories the results of which were reported.

LABORATORY TESTS

Laboratory studies as detailed below were carried out in order to demonstrate the safety of Ingelvac CircoFLEX.

Safety of the administration of one dose

A very detailed presentation of the design and of the results of a study involving a laboratory safety test of a single dose and repeat dose of PCV2 vaccine in 2 week old pigs was provided. No vaccine related significant adverse reactions were recorded throughout the study. A transient increase of body temperature was always recorded in field studies 4 hours after vaccination, but the increase in body temperature lasted no longer than 24 hours. The unexpected death respectively of a small number of piglets in both the vaccine and control groups was established not to be related to the administration of neither the test nor reference materials. None of the selected performance parameters was affected by the vaccination. No injection site lesions were palpated at any time during the study. Microscopic evaluation of representative animals which were necropsied on days 7, 14 and 21 revealed only mild to moderate inflammation of muscle (comparable to the reactions observed in control animals). In two cases (vaccinated animals), a score of 3 and 4 was assigned. The results clearly indicated that Ingelvac CircoFLEX was well tolerated by vaccinated animals.

Safety of an administration of an overdose

The evaluation of the safety of Ingelvac CircoFLEX following an overdose administration of 4 ml of vaccine to piglets approximately 2 weeks of age in comparison to control animals receiving the same dose/volume of water for injection, has been carried out. A detailed presentation of the design and of the results of the study on laboratory safety test of the administration of an overdose in 2 week old pigs with PCV2 vaccine was provided.

Treated pigs either received a single overdose volume of 4 ml of vaccine or a placebo injection of 4 ml water for injection. Both the vaccine and the placebo were injected intramuscularly in the right side of the neck. Good tolerance of the vaccine was confirmed as no injection site reactions were observed or palpated in any of the vaccinated animals. Statistically, there were no significant differences in body temperature between the treatment groups at any time during the measurement period. In both groups, temperature increased slightly above the baseline after the administration of the respective treatment preparations. On SD+2 after vaccination, the temperatures were similar in both groups. In the following days the control group temperature remained above that of the vaccinated group. Mild clinical signs described as depression, anorexia, and in case of one vaccinated animal, vomiting, were recorded (in both groups) starting generally, 4 hours after vaccination. On SD+11 and SD+12, respectively, one animal in each treatment group was found dead but in neither case could the death be attributed to the treatment. Statistically, a significant difference was observed between vaccinates and controls, in favour of the controls, for the ADWG from SD-1 to SD+14 but no difference was observed between the groups by the end of the study on SD+28. All animals were sero-negative at the starting of the study. While control animals remained sero-negative throughout the study (thus validating the study), by SD+14, in all but one of the vaccinated piglets an immune response was elicited by the vaccination. By SD+28 all vaccinates had high titres. The results show that an intramuscular administration of a 4ml dose of vaccine in piglets of the minimum age for vaccination was well tolerated in terms of local and systemic safety.

Safety of the repeated administration of one dose

Results were comparable with those obtained in the one dose safety study. All vaccinated animals showed a clear serological response when tested on SD+49, whereas no antibodies were detected in control animals, thus validating the study. The safety of the repeated administration of one dose was established. No significant difference was observed at any time between vaccinated and controls concerning rectal temperature, abnormal clinical signs or weight gain nor were any injection site reactions observed or palpated throughout the study. Again, a low number of deaths occurred in both groups but this was not related to the administration of the vaccine.

Examination of reproductive performance

No safety or efficacy data have been generated in breeding animals and no specific studies were performed in pregnant gilts or sows or lactating animals. The vaccine should not be used in pregnant or in lactating animals.

Examination of immunological functions

An overview of critical points relating to the immune response to infection, elicited in particular in young animals by the vaccination against PCV2, was provided and the role of passively acquired antibodies and the correlation between antibody titres and protection have also been addressed.

In order to mimic the widest range of field conditions, laboratory and field safety studies were carried out using CD/CD PCV2 antibody negative piglets, or piglets having varying levels of maternally derived antibodies (from low to moderate to high; sero-negative animals were considered piglets showing an antibody titre of less than 1:100), respectively. In general, no adverse impact due to the stringent experimental conditions on the outcome of the immune response elicited by the vaccination could be predicted. Although the impairment of the immune system in the context of PCV2 infection and vaccination is still a controversial issue, the potential impact of administering a large amount of vaccine antigen to host animals, with moderate to high level of antibodies, was addressed by *post mortem* investigations of kidneys (high serum antibodies against PCV2 in affected animals in association with immunoglobulins and immuno-complex deposition in the glomerula were predicted to be one of the most relevant factors triggering PDNS (Porcine Dermatitis and Nephropathy Syndrome) in two field studies. Results obtained from these studies appear to exclude any impact of the vaccine in triggering abnormal activities of the immune system, potentially leading to clinical expression of the disease.

Study of residues

No specific residue study has been carried out. This is acceptable due to the nature of the product (an inactivated, protein-based, preservative-free vaccine, formulated as an aqueous suspension not containing an oily adjuvant). In particular the carbomer polymer used as an adjuvant is out of scope of MRL regulations. Moreover, while carrying out field trials the presence of any residue of the vaccine at the injection site was investigated and in none of the animals submitted to necropsy could any residue of the vaccine be observed at the injection site. Consequently a withdrawal time of zero days is justified.

Interactions

No information is available on the compatibility of this vaccine with any other. Therefore the safety and efficacy of this product when used with any other (either when used on the same day or at different times) has not been demonstrated.

FIELD STUDIES

According to EU licensing requirements, the safety of the vaccine was further assessed in field conditions. Three field trials were conducted under swine farm management and breeding conditions typical of the European pig industry. These GCP (Good Clinical Practice) field trials were randomised, blinded and negatively controlled. Pre-screening tests carried out on sows as representative serological herd profiles at different times before study initiation, and on suckling piglets, revealed that none of the selected farms had a PCV2 negative antibody status. However, no viraemic animal was found upon pre-screening and no clinically apparent form of PCV2 infection was reported on any of the farms before study initiation. The presence of low (<1:100) moderate (1:100<T<1:1000) to high (>1:1000) PCV2 antibodies was considered indicative of the presence of a subclinical PCV2 infection within the selected herds. Various criteria were adopted for assessing the safe use of the vaccine under field conditions, including examination of the injection sites (local reactions), examination of general health status (systemic reactions), performance parameters, viraemia and PCV2 antibody titre.

Field study 1:

A detailed presentation of the design and of the results of this study was provided. The results showed that the vaccine was well tolerated in terms of local and systemic safety: no local, macroscopic or microscopic visible site reaction was ever reported throughout the study; no significant difference could be established on any day between the two groups in respect of the overall frequency of general health parameters or the severity of clinical findings. The higher increase in the mean body temperature registered in vaccinated animals 4 hours after vaccination did not result in any concern due to the transient nature and the absence of any changes in the general health status of the animals. No further concern for the safety of the vaccine derived from any of the remaining evaluation criteria.

No negative impact of the vaccination on major performance parameters represented by body weight was noted. By contrast, although not statistically significant, a better body weight and ADWG (Average Daily Weight Gain) was reported in vaccinated animals. No viraemia was detected throughout the study period.

Field study 2:

A detailed presentation of the design and of the results of this overdose study was provided. The results showed that the vaccine was well tolerated in terms of local and systemic safety. The nature as well as the onset and duration of local reactions were considered as not depending on the vaccine. Moreover, the incidence and type of findings were similarly reported for both vaccinated and control animals, making a correlation between vaccination and local observations highly unlikely.

While no statistically significant difference in the body weight was recorded between the two groups of animals at inclusion, a statistically significant difference was calculated between the treatment groups (in favour of the vaccinated animals) when comparing the average body weight gain and the ADWG of all animals between the first treatment and the end of fattening. Moreover, while no viraemia was detected throughout the study period in vaccinated animals, exposure to PCV2 field infection was confirmed. Indeed, PCV2 DNA was detected by qualitative PCR on SD+55 in 22% of control pigs, the onset of viraemia being correlated with the rise in antibody titres against PCV2 in these animals. During the first 4 weeks of the study, the decline of the maternally derived PCV2 antibody titre was similar in both treatment groups. The titre increased in the vaccinated group after the last treatment until the beginning of the fattening period. During the fattening period, the PCV2 antibody titres in vaccinated animals declined and were maintained at a low level until the end of the study (SD+155).

Field Study 3:

A detailed presentation of the design and of the results of this overdose study was provided. The data obtained from this study were in line with those obtained from a similar EU study (field study 2). Local reactions were observed in both treatment groups with a very low frequency. Clinical impairment of the general health status was found in animals of both groups without statistically significant difference and was associated with the specific disease situation on the farm (presence of *E. coli*, PRRSV and swine influenza) or to trauma. The higher increase of mean body temperature registered in vaccinated animals 4 hours after administration of the vaccine was significantly different in comparison to control group. Nevertheless the increased temperature was only transient and decreased to physiological values within 24 hours. Any negative impact of the vaccination on the major performance parameter represented by the measurement of body weight was noted. By contrast, although not statistically significant, a better weight gain was reported in vaccinated animals from the beginning until middle of the fattening period during which a field infection was confirmed. The better performance of vaccinated animals was explained with a strong reduction of viraemia, which indeed was more prominent and persisted longer in the control animals. Antibodies elicited by vaccination were also considered to have possibly played a role in the better performance against field infection.

Conclusion of field safety studies

In the field trials, the safety profile of the standard vaccine batch was clearly demonstrated. The SPC section adequately reflects the major outcome of a potential increase in body temperature observed after a few hours following vaccination.

ENVIRONMENTAL RISK ASSESSMENT

A satisfactory Phase I assessment of risk for this inactivated vaccine, in accordance with EMEA/CVMP/074/95, was provided. 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. Ingelvac CircoFLEX is judged to present no risk to the environment.

User safety

The following considerations are relevant:

- the vaccine is inactivated, to be injected intramuscularly; the vaccine is controlled for sterility. Therefore, from a microbiological point of view no risk for the operator is present.
- Carbomer is not within the scope of EU Regulation 2377/90 and therefore no MRL is required. It is not eye-irritant nor does it cause skin irritation or sensitisation.
- Due to the liquid nature of the vaccine, exposure may unintentionally occur by spillage of the vaccine on the skin or accidental self-injection. Accidental self-injections may cause mechanical irritation dependant on the localisation (e.g. injection in joints). Safety data raised in laboratory and field trials indicate a good local tolerance in the target animal.

Therefore, it was agreed that there is no specific risk associated with the nature of the product, its preparation and use.

RESIDUE ASSESSMENT

MRL

Sodium chloride is included in Annex II of Council Regulation (EEC) No. 2377/90.
Carbomer is considered not within the scope of Council Regulation (EEC) No. 2377/90.

Withdrawal period

Zero days.

OVERALL CONCLUSIONS ON SAFETY AND RESIDUES

The safety of the proposed vaccination scheme was supported. Potential risks from the use of the vaccine were tested in two laboratory trials and three field studies. Safety was demonstrated to be in compliance with current requirements. Laboratory safety data were presented and were deemed to be satisfactory to support the final formulation used.

The SPC wording reflects the conclusions regarding temperature increases arising from use of the product. No safety or efficacy data have been generated in breeding animals and no specific studies were performed in pregnant gilts or sows or lactating animals. The vaccine should not be used in pregnant or in lactating animals and this is reflected in the SPC which states “Do not use during pregnancy and lactation”. The safety studies presented 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 specific residue studies were carried out but a withdrawal period of zero days has been justified.

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

4. EFFICACY ASSESSMENT

Justification was provided to support the development of a vaccine against PCV2 infection. Relevant information has been provided in relation to the pathogenesis of the infection and to the complex nature of PCV2 associated diseases. Three laboratory and four field studies were carried out in order to investigate the efficacy of the vaccine under the conditions established by Directive 2001/82/EC (as amended by Directive 2004/28/EC), and Ph. Eur. general chapter 5.2.7.

In all studies, the dose volume of 1 ml was administered to each animal. With the exclusion of the Minimum Immunising Dose MID study ("the dose"/response study which was performed in order to enable a direct correlation of the varying antigen content with the response in the animals), the dose used in the efficacy studies was of the minimum expected potency (RP=1 as determined in MID study). The analytical methods used in the efficacy trial included all the histology and immunohistochemistry (IHC), virus isolation, qualitative and quantitative Polymerase Chain Reaction (PCR), and Immunofluorescent Antibody Test (IFAT). In addition, qualitative PCR was used and validated for detecting PCV2 in nasal swabs. Reference studies were provided and test methods were considered reliable. The choice of the challenge model was reasonable and scientifically acknowledged. A similar challenge model has been widely used in several vaccination/challenge experiments reported in referenced literature, and proven to be adequate.

LABORATORY TRIALS

In order to:

1. demonstrate that vaccination of pigs at 2 weeks of age or older:
    a. can reduce mortality, clinical signs and lesions of acute and chronic Porcine Circovirus Disease (PCVD)
    b. can prevent effect of PCVD on the performance of pigs such as weight loss, growth variability and increased time to slaughter;
2. support the evidence that, in addition to these claims, vaccination can reduce PCV2 excretion, viral load and virus persistence in the body.
3. demonstrate that onset of protection occurs as early as 2 weeks post vaccination and lasts for at least 17 weeks.

three experimental studies were carried out aiming

1. to establish the minimum immunising dose (MID) capable of guaranteeing the level of claimed efficacy
2. to confirm:
    a. a 14 day onset of Immunity (OOI)
    b. a 4 month duration of Immunity (DOI)

Determination of the Vaccine Dose

Concerning minimum immunising dose (MID) a presentation of the design and the results of a study on evaluation of MID of PCV2 killed baculovirus vector following challenge with a virulent strain of PCV2 was provided.

In this study, all 3 vaccine inclusion levels (Relative potencies of respectively 0.05, 0.46 and 1) provided significant levels of protection against a PCV2 challenge carried out 32 days after a single vaccination, based on the following primary parameters: lymphoid depletion, lymphoid inflammation and lymphoid tissues positive for PCV2 by IHC staining as well as reduction of duration of nasal shedding. Reduction of loss of weight gain (secondary parameter) was achieved in the groups 1 (RP of 0.05) and 3 (RP of 1).

No dose-response relationship was clearly established. The best efficacy results were obtained with the highest dose tested (RP = 1) and so this was selected for the further efficacy trials.

Onset of protection

The design and results of a study on evaluation of OOI of PCV2 killed baculovirus vector, following challenge with a virulent strain of PCV2 were provided. A single dose of the vaccine (RP = 1) was shown to significantly reduce all the relevant parameters that can be induced by the applied PCV2 experimental challenge 2 weeks after vaccination (presence of characteristic lesions in lymph nodes and presence of PCV2 within these lesions, as well as pyrexia and reduction of body weight gain). At the time of challenge, Geometric Mean Titres (GMTs) were significantly higher in vaccinated animals than in placebo animals. After challenge, GMTs in placebo animals were significantly higher than in vaccinated animals. The onset of immunity of 2 weeks was thus established.

Virus nasal shedding was only assessed qualitatively and no reduction of the proportion of vaccinated animals shedding PCV2 was evidented in this study, but a significant reduction of the duration of nasal excretion. The quantitative reduction of nasal excretion was questioned and further argumentation was given on this issue. Given that a significant reduction of the duration of excretion was demonstrated in the 3 laboratory studies and a significant reduction of the amount of virus excreted was consistently achieved within the 4 field efficacy studies, a claim of reduction of nasal shedding was accepted.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

The evaluation of the potential interference of maternally derived antibodies on the efficacy of vaccination in 2 weeks piglets was deferred to field studies.

Duration of Immunity

A presentation of the design and results of a study on evaluation of DOI of PCV2 killed baculovirus vector, following challenge with a virulent strain of PCV2 at approximately 3 and 4 months post-vaccination was provided.

The challenge carried out 3 months after vaccination was not sufficiently virulent and no conclusion could be drawn from this part of the study. The second part of the study demonstrated a duration of protection of 4 months: a single dose of the vaccine (RP = 1) was shown to significantly reduce the lesions in lymph nodes and the presence of PCV2 within these lesions, as well as the reduction of body weight gain induced by a PCV2 challenge applied 4 months after vaccination. A significant reduction of the duration of excretion was also demonstrated. GMTs were significantly higher in vaccinated animals compared to placebo animals from 4 weeks post vaccination until the time of challenge). After challenge, GMTs were significantly higher in placebo than in vaccinated animals.

In light of the data provided, it is possible to state that under the established experimental conditions, efficacy of one RP=1-based dose of standard reference vaccine was shown in terms of reduction of lymphoid depletion and inflammation caused by PCV2 infection. In addition, supportive evidence in terms of reduction of virus load in lymphoid tissues and reduction of the mean duration of PCV2 nasal excretion was provided. Efficacy of the vaccine was shown from 2 weeks after vaccination up to 4 months.

FIELD TRIALS

According to EU licensing requirements, and either in order to support efficacy parameters of the vaccine already tested under experimental conditions or to be further assessed under field conditions, four field trials were conducted under swine farm management and breeding conditions representative of the European pig industry. In this respect, the primary efficacy variable was defined by the ADWG measured at the middle of the fattening period; secondary efficacy variables were estimated in a range of additional clinical findings (such as bodyweight gain measured at different time intervals, frequency of 'runts'), including the impact of economical parameters (such as the average time from weaning to slaughter), the occurrence of clinical signs, the onset, duration, end and number of days

positive for viraemia, the quantification of viral load and the proportion of viraemic animals measured by real-time PCR assay.

Presentations of the design and of the results of four studies in different EU countries were provided. Reduction of viraemia (proportion of animals presenting viraemia, duration of viraemia, viral load in the blood) was consistently achieved, as well as reduction of weight losses up to the end of the fattening period in the context of PCVD (and reduction of growth variability and increased time to slaughter). Reduction of frequency of runts at the end of fattening period was demonstrated in the two field studies with evidence of PMWS. Reduction of mortality was consistently recorded in face of acute PCVD. As onset of viraemia occurred 17 weeks after vaccination in one field study (and vaccinated animals were protected), the latter study confirms the duration of protection of 4 months as demonstrated in laboratory conditions and the claimed duration of protection of 17 weeks is acceptable.

In each of the four field efficacy studies, it was demonstrated that vaccination was able to reduce the clinical signs associated with PCV2 related diseases (PCVDs). In such cases where the expression of the disease was severe, and the mortality rate in the control animals was high, the reduction of clinical signs became significant. Quantitative reduction of PCV2 nasal shedding was originally demonstrated in one field study. A further analysis demonstrated that virus nasal shedding was quantitatively significantly reduced in the three other field studies. Finally, the field studies have shown that vaccination was not negatively influenced by the presence of MDA: in each field study, vaccinated animals which had high titres of MDA at the time of vaccination showed significantly reduced viraemia as well as reduced weight losses compared to placebo animals having high titres of MDA. Vaccinated animals having high MDA titres at the time of vaccination showed similar reduction of weight losses as vaccinated animals which had low MDA titres.

OVERALL CONCLUSION ON EFFICACY

Three laboratory and four field studies were carried out in order to investigate the efficacy of the vaccine against acute and chronic (subclinical) form of PCVD. In all studies, the dose volume of 1 ml was administered to each animal. In light of the data provided, it is possible to state that under the established experimental conditions, efficacy of one RP=1-based dose of standard reference vaccine was shown in terms of reduction of lymphoid depletion and inflammation caused by PCV2 infection. In addition, supportive evidence in terms of reduction of virus load in lymphoid tissues and reduction of the mean duration of PCV2 nasal excretion was provided. Efficacy of the vaccine was shown from 2 weeks after vaccination up to 4 months.

In field trials reduction of viraemia (proportion of animals presenting viraemia, duration of viraemia, viral load in the blood) was consistently achieved, as well as reduction of weight losses up to the end of the fattening period in the context of PCVD (and reduction of growth variability and increased time to slaughter). Reduction of mortality was consistently recorded in face of acute PCVD. Data were provided which supported the claimed duration of protection of 17 weeks.

The claim agreed is “For active immunisation of pigs, over the age of 2 weeks, against Porcine Circovirus type 2 (PCV2) to reduce mortality, clinical signs - including weight loss - and lesions in lymphoid tissues associated with PCV2 related diseases (PCVD). In addition, vaccination has been shown to reduce PCV2 nasal shedding, viral load in blood and lymphoid tissues, and duration of viraemia.”

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

V. RISK-BENEFIT BALANCE

Ingelvac CircoFLEX is a biotechnology derived inactivated immunological veterinary medicinal product intended to be used for the active immunisation of pigs against acute and chronic forms of Porcine Circovirus Disease (PCVD). Ingelvac CircoFLEX administered once via the intramuscular route to pigs from two weeks of age is capable of reducing mortality, clinical signs (including weight loss) and lesions in lymphoid tissues associated with PCV2 related diseases (PCVD) in addition to a reduction of PCV2 nasal shedding, viral load in blood and lymphoid tissues, and duration of viraemia.

The data submitted confirmed the acceptability of the proposed formulation and presentations, the suitability of the specification for the active substance, the method of manufacture of the product and the validity of the test methods applied to the product. The stability data provided for the finished product justify a shelf life of 15 months.

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

All safety studies were conducted with a vaccine batch formulated to contain the maximum Relative Potency. Following the administration of one dose, a transient increase of body temperature was always recorded in field studies 4 hours after vaccination, but the increase in body temperature lasted no longer than 24 hours.

With an overdose administration of vaccine to piglets approximately two weeks of age compared to control animals, mild clinical signs described as depression, anorexia, and in case of one vaccinated animal, vomiting, were recorded (in both groups) starting generally, 4 hours after vaccination. Local tolerance was acceptable.

Concerning the repeated administration of one dose, results were comparable with those obtained in the one dose safety study.

With regard to reproductive performance no safety or efficacy data have been generated in breeding animals and no specific studies were performed in pregnant gilts or sows or lactating animals. The vaccine should not be used during pregnancy and lactation.

Due to the nature of the product, no residue depletion study has been carried out. While carrying out field trials the presence of any residue of the vaccine at the injection site was investigated and in none of the animals submitted to necropsy could any residue of the vaccine be observed at the injection site. A withdrawal time of zero days is justified.

The safety of the vaccine was further assessed in three GCP field trials conducted under swine farm management and breeding conditions typical of the European pig industry. In these field trials, the safety profile of the vaccine was clearly demonstrated and the SPC adequately reflects the major outcome of a potential increase in body temperature observed after a few hours following vaccination.

Concerning potential risks posed to the environment, it was concluded that the use of product does not present a risk to the environment.

Concerning the user safety, it was agreed that there is no specific risk associated with the nature of the product, its preparation and use.

Three laboratory and four field studies were carried out in order to investigate the efficacy of the vaccine. In the laboratory studies the efficacy of one RP=1-based dose of standard vaccine was shown in terms of reduction of lymphoid depletion and inflammation caused by PCV2 infection. In addition, supportive evidence in terms of reduction of virus load in lymphoid tissues, reduction of the mean duration of PCV2 nasal excretion and reduction of loss of weight gain was provided. Efficacy of the vaccine was shown from 2 weeks and up to 4 months after vaccination.

In order to support these efficacy parameters four field trials were conducted under swine farm management and breeding conditions representative of the European pig industry. Reduction of viraemia (proportion of animals presenting viraemia, duration of viraemia, viral load in the blood) was consistently achieved, as well as reduction of weight losses up to the end of the fattening period in the context of PCVAD (and reduction of growth variability and increased time to slaughter). Reduction of frequency of runts at the end of fattening period was demonstrated in the two field studies with evidence of PMWS. Reduction of mortality was consistently recorded in face of acute PCVD. As onset of viraemia occurred 17 weeks after vaccination in one field study (and vaccinated animals were protected), the latter study confirms the duration of protection of 4 months evidenced in laboratory conditions.

In each of the four field efficacy studies, it was demonstrated that vaccination was able to reduce the clinical signs associated with PCV2 related diseases (PCVDs). In such cases where the expression of the disease was severe, and the mortality rate in the control animals was high, the reduction of clinical signs became significant. PCV2 nasal shedding was quantitatively significantly reduced in the four field studies. Finally, the field studies have shown that vaccination was not negatively influenced by the presence of MDA (maternally derived antibodies).

Based on the 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.