

SCIENTIFIC DISCUSSION

Product name:	BAYOVAC CSF E2
Procedure No.:	EMA/V/C/053/01/0/0
Marketing Authorisation Holder :	BAYER AG Business Group Animal Health D-51368 Leverkusen Germany
Active substance:	Classical Swine Fever Virus (CSFV) - E ₂ subunit antigen
Pharmaceutical form:	Emulsion for injection
Strength:	Minimum 32 µg of E ₂ glycoprotein
Presentation:	Multidose glass vials containing 20 ml (10 doses) (packed as 54 vials in 1 styrofoam box), 50 ml (25 doses) (packed as 35 vials in 1 styrofoam box), 100 ml (50 doses) (packed as 12 vials in 1 styrofoam box) and 250 ml (125 doses) (packed as 10 vials in 1 styrofoam box), closed with rubber stoppers and an aluminium seal
Target species:	Pigs
Withdrawal period:	Zero days
Route of administration:	Intramuscular injection
Product type:	Immunological
Therapeutic indication:	Active immunisation of pigs, over the age of 2 weeks, against Classical Swine Fever (CSF), to prevent mortality, reduce clinical signs of the disease and the excretion of field virus.

3.1. Introduction

Bayovac CSF E2 is an emulsion vaccine containing as the active component a minimum of 32 µg of classical swine fever virus (CSFV) E2 antigen (subunit vaccine) per dose of 2 ml.

The indication for BAYOVAC CSF E2 is active immunisation of pigs, over the age of 2 weeks, against Classical Swine Fever (CSF), to prevent mortality, reduce clinical signs of the disease and the excretion of field virus.

By using only the immunodominant E2 antigen of CSFV in the vaccine, vaccinated animals will only develop an immune response against E2, including antibodies to E2. In contrast animals infected with field virus will develop antibodies to all immunodominant antigens of CSFV e.g. E2, E^{RNS} and NS3.

The volume of a single dose is 2 ml to be injected by the intramuscular route. The basic vaccination consists of 2 doses, the second dose being given after a 4-6 weeks interval. Revaccination with a single dose is recommended at 6-month intervals. For fattening pigs over the age of 10 weeks and in emergency situations one intramuscular inoculation with one dose is administered for basic immunisation.

The CSFV-E2 antigen is produced in an insect cell baculovirus expression system. The baculovirus has been genetically modified to introduce the CSFV gene encoding the CSFV-E2 antigen. The E2 antigen is secreted in the antigen production medium.

Prior to the assessment a meeting was held with a representative of DG SANCO of the European Commission.

There were three main conclusions resulting from the discussions during this meeting:

1. For obvious epidemiological reasons the company is not due to produce results on efficacy from field trials; only safety field trials were authorised by the competent authorities;
2. BAYOVAC CSF E2 being a subunit vaccine at least two antigens may be chosen as markers. Therefore several diagnostic companies apart from the vaccine manufacturer may introduce on the market different diagnostic tests; consequently, the company has only to provide scientific evidence that the product works effectively as a marker vaccine;
3. The use of such a vaccine must be authorised by DG SANCO competent authorities. It was therefore agreed that the CVMP would only give an opinion on the quality, safety and efficacy aspects of the product (in accordance with Council Directive 81/852/EEC); DG SANCO would decide if, for epidemiological purposes, it needs further data for their behalf, from the company, on the basis of the CVMP assessment report.

II. OVERVIEW OF PART II OF THE DOSSIER: ANALYTICAL ASPECTS

The main quality issues specific for this vaccine are:

- the novel baculovirus/insect cell production system
- the genetic development of the host cell/vector system
- the potency of the vaccine
- the stability of the antigen in the water-in-oil adjuvant suspension

A. QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

A.1. Composition

	Names of ingredients	Quantity per <u>2 ml</u> (= 1 dose)	Function	Reference
Active ingredient	E2 glycoprotein of Classical Swine Fever Virus (CSFV)	min. 32 µg	Induction of immunity	In-house specification

Medium buffer	Medium buffer		from production medium	In-house specification
Adjuvant	Marcol 52 & Montanide 80		Oil phase of adjuvant	Ph Eur & in-house specification
Adjuvant	Montanox 80 (= Tween 80)		Emulsifier	Ph Eur
Buffer	Phosphate Buffered Saline		Adjuvant buffer	In-house specification
Preservative	Thiomersal	≤ 0.2mg	antimicrobial preservative	Jap. Ph. 94

A.2. Presentation

20, 50, 100 or 250 ml Type I (Ph Eur) glass vials, corresponding to 25, 50 or 125 doses of 2 ml, closed with grey bromobutyl rubber stoppers (Ph Eur) and sealed with aluminium seals with flip off caps.

A.3. Preservative

Thiomersal ≤ 1/10 000 (= ≤ 0.2 mg/dose). The concentration of thiomersal in the vaccine is as generally used in other vaccines.

As no data were presented to prove the preservative efficacy of thiomersal, the Applicant committed to provide data proving the preservative efficacy of thiomersal before 15 November 2000. These data were received on 15 November 2000 and are currently under evaluation by the Rapporteur.

A.4. Development Pharmaceuticals

A.4.1. Choice of the vaccine antigen

The E2 structural protein was chosen as the vaccinating antigen because it is an immunodominant antigen and antibodies to E2 are strongly virus neutralizing and were shown to be protective.

Furthermore, the use of monovalent E2 antigen allows the development of a marker vaccine provided that appropriate tests for differentiation are available.

A.4.2. Choice of the inactivation method

Live baculovirus is inactivated using 2-bromoethyl-imminebromide.

A.4.3. Adjuvant selection

A water-in-oil-in-water emulsion, i.e. a double emulsion, is used in the vaccine as an adjuvant. This adjuvant was examined, in comparison with two other adjuvants («Bay 1» & «Bay 2»), with regard to efficacy in generating an immune response to E2. The double emulsion was found to be superior to the other two adjuvants examined.

The adjuvant is known to cause local tissue irritation and lesions, however considering the importance of the disease, the benefits of the vaccination should outweigh the risk and such lesions are therefore considered acceptable (see also Safety section C1-C4).

A.4.4. Antigen dose, vaccination schedule

From a dose titration study it was estimated that a single dose of 28.35 µg E2 antigen in vaccine formulation protected 95% of vaccinated animals from an experimental challenge and was efficacious in the prevention of horizontal and transplacental transmission. The product specification is $\geq 32 \mu\text{g}/2 \text{ ml}$ single dose.

The minimum antigen quantity which was estimated to be 95% protective was 2 µg. Therefore it is felt that the minimum specification of 32 µg will provide an adequate level of protection whilst remaining safe.

A.4.5. Preservative

Thiomersal at a concentration of $\leq 1/10\ 000$ ($= \leq 0.2 \mu\text{g}/\text{dose}$) is used as a preservative.

4.6. Choice of the baculovirus expression system and of the insect cell line for baculovirus propagation

The choices of the baculovirus expression system and of the insect cell line for baculovirus propagation have been adequately justified.

B. METHOD OF PREPARATION

B.1. & 2. Method of preparation

The Production Flow Chart has been commented on adequately by the applicant. The vaccine is currently manufactured at the ID-Lelystad site (in The Netherlands). Production of the E2 subunit antigen is based on fermentation of *Spodoptera frugiperda* cells (Sf21) infected with the recombinant baculovirus vector. The antigen is secreted into and harvested from the production medium, processed and 2-bromoethyl-imminebromide treated for virus-inactivation. A first water-in-oil emulsion is prepared containing the antigen. This emulsion is subsequently mixed with a second water phase to yield the final bulk. The quantity of antigen for formulation is standardised, based on a capture ELISA antigen determination before addition of the adjuvant.

Sf-21 production cell cultures are prepared by serial passages from the Master Cell Bank and virus harvest is prepared following serial passages from the Master Seed Virus. Medium free from antibiotics and serum is used for cell expansion and virus replication.

B.3. Validation studies

The influence of the different parameters on the production process was validated. The inactivation process has been validated and adequately described by the applicant.

The consistency of the production process was demonstrated by 5 consecutive runs of production of inactivated antigen and formulation of 3 independent vaccine batches including filling in respective subbatches.

The identity of the E2 is determined in the final product by SDS-Page and immunoblot. The content of the E2 in the final product is checked but only informative.

The formulation equipment currently in use has been fully validated.

B.3.1. Validation of the virus inactivation

Inactivation is performed with BEI (2- bromoethylimmine-bromide). Inactivation with ethylenimmines, e.g. BEI is rapid with first order kinetics, which enables extrapolation.

The inactivation method involves a defined final concentration of Binary Ethylenamin in the cell free virus fluids, which are incubated under constant stirring. Bovine virus diarrhoea, Infectious Bovine Rhinotracheitis virus, Foot and Mouth Disease virus and parapoxvirus ovis have been shown to be completely inactivated under these conditions.

Inactivation kinetic studies on production scale batches were not feasible due to the fact that after microfiltration the virus titre is reduced to levels of $\pm 10^1$ pfu/ml. However, the validation study in laboratory and in production scale shows that the employed inactivation process leads to a complete inactivation of the baculovirus within <67% of the minimum inactivation time.

B.3.2. Validation of the quantitative ELISA for determination of the E2 antigen

The method is a classical ELISA using a characterised purified reference preparation. A suitable validation has been performed.

B.3.3. Validation of the baculovirus titration

The method is used at four production steps to verify the yield of virus before and after microfiltration and for monitoring the inactivation process, before and after inactivation.

C. STARTING MATERIALS

C.1. Listed in a Pharmacopoeia

Dimethyl sulfoxide (DMSO), Gentamicin, Hydrochloric acid, Polysorbate 80 (=Montanox, Tween 80), Sodium hydroxide, Sodium thiosulphate, water for injection, and Marcol 52 are stated to comply to the appropriate Ph. Eur. monographs and thimerosal complies with the Japanese Pharmacopoeia.

In view of the aseptic production process, the microbial purity of the starting materials listed has been controlled. It has been confirmed that there is no material of animal origin used in the preparation of polysorbate 80.

C.2. Not listed in a Pharmacopoeia

C.2.1. Starting materials of biological origin

The following starting materials are of biological origin and deserve special attention:

- 1) recombinant baculovirus
- 2) Sf-21 insect cell line
- 3) Foetal calf serum

C.2.1.1. Seed materials

- 2.1.1.a Recombinant *Autographa californica* Multiple Nuclear Polyhedrosis Virus expressing the CSFV-E2 antigen

The original recombination is summarised as follows:

Autographa californica virus (AcNPV) and recombinant viruses were propagated in the *Spodoptera frugiperda* cell line, SF21. The cells were grown as monolayers and for cotransfection in suitable medium.

A swine kidney cell SK 6 was used to propagate HCV strain Brescia. SK 6 cells were grown in Eagle basal medium with 5% foetal bovine serum and antibiotics.

The gX signal sequence was constructed by annealing a 3' stretch of 7 complementary nucleotides of a 51-mer oligonucleotide and a 49-mer oligonucleotide and by filling in the 5' single strands with *Taq* polymerase (using the PCR technique). The *Bam*HI and *Bg*/II flanking sites (included in the sequence of the 49-mer and the 51-mer, respectively) were digested and the *Bam*HI-*Bg*/II fragment was isolated from an agarose gel by electroelution. The fragment was ligated into *Bam* HI site of the pAcAS3 vector and ligation mixtures were used to transform *E. coli* DH α cells by electroporation with a proprietary Gene pulser to give pAcAS3gX with a unique *Bam*HI site. Plasmid DNA was recovered by standard methods and was purified by CsCl centrifugation.

DNA fragments encoding E2 with and without TMR were obtained by polymerase chain reaction with a cDNA clone of HCV strain Brescia VP6 as template, in a 37 cycle amplification with *Taq* polymerase. Noncoding, flanking *Bam*HI sites were included in the sequence of the forward and reverse primers used to amplify the E2 fragments. The polymerase chain reaction products were *Bam*HI digested, and the E1 fragments were isolated from an agarose gel by electroelution and ligated into *Bam*HI site of the pAcAS3gx vector to give pAcAS3gx.E2 with and without TMR.

In this and in previous experiments, expressing the E2 glycoprotein of CSF in the baculovirus system, it was shown that the version of E2 encoded without a C-terminal membrane anchor i.e. transmembrane region, TMR, was expressed in insect cells and medium, in significantly higher quantities than E2 encoded with TMR. This vaccine is based on the E2 glycoprotein encoded without TMR. The E2 without TMR is therefore only addressed in this review.

For the transfection and selection of baculovirus recombinants expressing E2, confluent monolayers of SF21 cells were cotransfected with 1µg of wild type AcNPV DNA and 2µg of pAcAS3gX.E1 without TMR, by a calcium phosphate precipitation technique. Following cell growth, recombinant viruses expressing β-galactosidase were isolated from the medium using a plaque assay. These plaques were then used to infect monolayers of SF21 cells grown in M24 wells; after 4 days cells were fixed and immunostained to test for expression of E2. AcNPV recombinant virus expressing E2, were plaque purified three times.

To ensure that the DNA sequences encoding E2 were correctly inserted into the p10 locus of baculovirus, southern blots of restriction enzyme digested viral and cellular DNAs were hybridized with a probe of HCV DNA clone, VP6. It was demonstrated that the DNA sequences encoding E2 were correctly inserted in the p10 locus of the baculovirus.

The nucleotide sequence of the junction between E2 and simian virus 40 (SV40) terminator in the DNA of AcNPV recombinant viruses was determined by the dideoxy chain termination method with T7 DNA polymerase and E2 specific oligonucleotide primer, p428. The sequence at the 3' junction between the E2-TMR fragment and the vector showed that the E2 and the vector sequences were fused by ligation of a vector having a degraded *Bam*HI site and a blunt E2 fragment.

The E2 expression products observed in Bac E2[-] infected cells were further characterised by radio immunoprecipitation with a mixture of three E2-specific Mabs.

Furthermore, this Master Seed Lot has been tested according to the European Pharmacopoeia Monograph 0062 «*Vaccines for veterinary use*», and the Note for Guidance on extraneous agents (III/3427/93).

The MSV was tested for possible presence of retrovirus. No retrovirus could be found and the MSV is therefore considered to be free of retroviruses.

The MSV was tested for the possible presence of *Spiroplasma sp.* No *Spiroplasma sp.* could be detected in Master Seed Virus and Working cell stock. The MSV is considered to be free of *Spiroplasma sp.*

The MSV was tested for possible presence of *Brucella suis*. No *Brucella suis* could be detected in the MSV, which is therefore considered to be free of *Brucella suis*.

The company's rationale of performing general extraneous agents testing supplemented by specific testing for viruses of the target species is acceptable in view of the absence of specific regulatory guidance concerning insect cells.

The genetic stability of the construct has been demonstrated by comparing sequences of samples taken at the end of fermentation with the sequence in the Master Seed Lot.

Immunochemical characterisation of the E2 antigen produced by the recombinant baculovirus system is performed after each production run. It was accepted that monitoring for size and the presence of the two epitopes involved in induction of protection is sufficient to guarantee the immunogenicity of the E2 antigen.

C.2.1.1.b Sf-21 insect cell line and cell bank system

IPLB-SF21-AE is a *Spodoptera frugiperda* insect cell line which is used as the substrate to propagate the recombinant *Autographa californica* Multiple Nuclear Polyhedrosis Virus expressing the CSFV-E2 antigen.

Multiple passages were performed to adapt the cell line to suspended growth. The Master Cell Stock (MCS) and the Working Cell Stock (WCS) are stored in liquid nitrogen.

Testing of the MCS & WCS is performed according to the European Pharmacopoeia General text 5.2.4 «*Cell cultures for the production of veterinary vaccines, CELL LINES*» and the Note for Guidance on extraneous agents (III/3427/93).

Adequate information has been provided regarding the history of the Sf-21 cells and of the MCS.

The Master Cell Seed (MCS) was tested for the possible presence of retrovirus, and is considered to be free of retrovirus.

The Working Cell Seed was tested for the presence of *Spiroplasma sp* (the production is started from the WCS). No *Spiroplasma* could be detected in WCS. Therefore it is considered that the WCS and also the MCS are free of *Spiroplasma spp*.

C.2.1.1.c Foetal calf serum

Serum is only used as a cryopreservative for frozen cell stocks. The country of origin is restricted to New Zealand, Australia or United States of America.

Batches of serum are subjected to γ -irradiation with 25 and 42 kGy as an extra safety measure. The irradiation is adequately tested by the supplier or the vaccine manufacturer.

C.2.2. Starting materials of non-biological origin

The cell culture medium is a serum free medium. In-house specifications are used for sterility, endotoxins, pH, osmolarity, storage conditions and expiry date.

The complete qualitative and quantitative composition of culture media used for production has been provided.

D. IN-PROCESS CONTROL TESTS

A flow chart indicating the in-process controls at the different production steps of the E2 subunit vaccine has been submitted.

Each of the three phases of the double emulsion adjuvant has been tested for bioburden prior to sterile filtration.

In the production process no other starting materials of biological origin are tested other than the tested Seed Virus and Cell Seeds. There is no risk of contamination with extraneous agents from any biological source.

E. CONTROL TESTS ON THE FINISHED PRODUCT

A flow chart indicating the control tests on the finished product has been presented. The potency test deserves special attention:

The evaluation of vaccine potency is based on the titres in sera from the six pigs vaccinated with one, 2 ml dose of vaccine.

The principles of combining the innocuity test and a serological immunogenicity test have been found acceptable by the Committee. The serological specifications have been adequately supported.

II. F. STABILITY

II.F.1. Stability of the bulk antigen

Data on three lots of bulk antigen (inactivated and neutralised antigen solution) were submitted. Stability parameters were visual aspect, pH, E2 antigen concentration, protein pattern by SDS-PAGE and demonstration of V3 & V8 epitopes by immunoblotting.

After storage at $+5^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 12 months. No significant alterations or loss in antigen content as measured by ELISA were detected.

After storage at $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 12 months. No significant alterations or loss in antigen content as measured by ELISA were detected.

After storage at $40^{\circ}\text{C} \pm 3^{\circ}\text{C}$ for 28 days. No significant alterations or loss in antigen content as measured by ELISA were detected.

Real time stability (-20°C) were presented for 18 months storage time.

The antigen was still immunogenic after storage at the indicated temperature as demonstrated by the described immunisation experiment in pigs. Based on serology no differences were observed as compared with regular vaccines that were tested a year earlier formulated from the same intermediate product.

Physicochemical analysis of intermediate product under all conditions tested revealed no trends which may be expected to influence stability of the E2 antigen.

It was concluded from the data that the antigen was stable under all conditions tested.

The data presented show stability of the antigen following storage at -20°C for 18 months. It is considered to extend the storage time for the antigen under the proposed condition to 24 months.

II.F.2. Stability of the finished product

The proposed shelf-life of the finished product is 18 months at 2-8°C. Stability testing is still in progress. Data on six lots of finished product (three 50 ml & three 250 ml vials) have been submitted. Stability parameters were Visual aspect, pH, E2 identity (semi-quantitative antigen content after extraction), protein pattern by SDS-PAGE, demonstration of V3 & V8 epitopes by immunoblotting, droplet size, conductivity, phase separation test (P.S.T.) and viscosity.

After storage at 5°C ± 3°C 12 months. No significant alterations were detected with the exception of large fluctuations (mostly upwards) in viscosity.

After storage at 25°C ± 3°C 12 months. In a number of batches V8 could not be demonstrated at three months and a very weak protein pattern was noted.

After storage at 40°C ± 3°C for 28 days. Alterations in almost every parameter occurred at several time points.

Real time stability data (at 5°C) were reported over a period of 18 months. A potency trial in pigs was carried out after 14 months storage at 5°C. In this trial serological responses against CSF were measured 21 days post vaccination.

In addition the quality dossier mentioned vaccination/challenge studies on three vaccine batches stored for 12 and 21 months at 4-8°C. Full protection from challenge was observed, even at very low antigen doses.

The vaccine remains stable for at least 14 months, irrespective of the filling volume, as demonstrated in real time stability studies at 5°C.

The vaccine batches were stable even in accelerated and forced stability studies. Eventually degradation of the double oil emulsion could be observed visually after storage at + 25°C for 6 months and at +45°C for 14 days, respectively. Thereafter a clear phase separation between oil and water phases was observed. The original emulsion could not be restored, not even by vigorous shaking of the flasks. Measurement of viscosity and conductivity verified the above-described observation.

To determine the antigen content in final product an extraction method was employed. The antigen, in normal conditions, is present in small water droplets enclosed within oil droplet of approx. 10 µm in diameter. When the antigen is in the double oil emulsion epitopes that are recognised by monoclonal antibodies in the E2 quantitative ELISA are inaccessible. By extraction a part of the E2 antigen is released into the water phase enabling a determination of the antigen content in the quantitative E2 ELISA.

One study was also performed to determine the stability of the vaccine batches when stored accidentally under temperatures below the recommended storage temperatures (+2°C to +6°C). Three final product batches were used. The vaccines were stored for 24 hours at 0°C and at -20°C. Results show that the incubation at 0°C had no influence on the physicochemical and immunochemical characteristics of the vaccine batches. Freezing of the vaccine however resulted in a phase separation of the oil and water phase and should therefore be avoided.

Based on the results of the stability trials, no loss of potency is anticipated if vials are broached aseptically and stored as indicated in the package information.

In conclusion, a shelf-life of 14 months is recommended.

3. SAFETY ASSESSMENT (PHARMACO-TOXICOLOGICAL)

A. INTRODUCTION

The product is a subunit vaccine. In terms of safety there is no concern about the possibility of vaccine induced disease since the product contains no intact virus particles. However, the product is manufactured in an insect cell line and the baculovirus vector used contains a portion of the CSF genome. Therefore, in order to eliminate the possibility of recombination and/or reassortment with field virus the product is "inactivated" using binary ethylene imine. This treatment will destroy the nucleic acid structure of both the CSF gene and vector.

Consequently, assessment of safety is primarily a matter of vaccine tolerance. The product is adjuvanted using a double oil emulsion. The oils used in this formulation are mineral oils. Although these oils and the emulsifiers that are used with them are well established as effective adjuvants it is also well known that pigs do not generally tolerate mineral oils very well. Assessment of local reactions is therefore important.

Although conventionally bred animals have been used in some studies, all except one of the studies reported are laboratory based. Confirmation that these studies have been conducted in compliance with the principles of GLP as laid down in OECD guidelines is provided.

B. GENERAL REQUIREMENTS

All safety tests have been performed in the target animals. The majority of the studies reported were designed primarily as efficacy studies.

In general, safety studies should be conducted with material of the highest titre or potency. However, this product is manufactured to contain a specific quantity of the relevant antigen (E2) (consistency) and all batches are therefore suitable for the demonstration of both efficacy and safety.

It should be noted that some of these batches were manufactured using antigen produced by monolayer culture whereas the intended method of manufacture is in suspension culture. Equivalence of these methods is more important in terms of efficacy and in order to address the question the Applicant has performed specific studies to enable comparison between the methods. For the purposes of evaluating safety, a comparison of all conducted studies showed that the change from monolayer to fermenter production has no effect on the safety of the final product. Therefore, all of the data provided may be considered relevant to the intended product.

C. LABORATORY TESTS

C.1. Safety of the administration of one dose

Seven efficacy studies have been submitted. Unfortunately, they do not directly contribute to the evidence of safety of the final product because the requirements of Council Directive 81/852/EEC are not fully fulfilled. In none of these studies the local reactions have been recorded. Detailed records on systemic reactions are generally lacking. Moreover, one or more of the following criteria were lacking: the amount of antigen is lower than that of normal potency of the product; the observation period was lower than 14 days; the systemic reactions were not recorded; macroscopic and/or microscopic examinations were not performed.

All these studies were performed on 6-7 week old piglets. No information on animals of the minimum age of administration (2 weeks old piglets) and on pregnant sows is provided. Therefore, the safety of the product has not been tested in each category in which it is intended for use. Six of these studies are reported more extensively in Part 4.

The first study cannot support the safety of administration of one dose as the maximum amount of antigen used is 16 µg, whereas the applicant recommends the administration of 32 µg. However, side effects of a slight reduction in feed uptake, mild diarrhoea and a transient increase in rectal temperature are reported that contribute to the evaluation of safety.

In a second study, 26 x 6-7 week old SPF piglets divided into 3 groups of 6 vaccinates (doses: 2 µg, 8 µg and 32 µg) and one group of 2 controls were used. Two additional sentinel animals were included in each group of vaccinates. Control animals were vaccinated with 2ml of adjuvant only but the sentinels remained unvaccinated. Mild diarrhoea, anorexia and depression were recorded 3 days after vaccination in groups A (vaccinated with a dose of 2 µg) and C (vaccinated with 32 µg), and one animal in group A apparently vomited regularly throughout the observation period. Body temperature in animals administered adjuvant and 2 µg of antigen remained within normal ranges. In contrast, a number of animals administered higher doses showed a slight, transient increase in temperature immediately following vaccination. Only one animal displayed a temperature > 40.5°C and this occurred in a sentinel (unvaccinated) where 40.8°C was recorded on one day. Rectal temperatures were measured only after vaccination and never before. Therefore the assessment of the effect of vaccination on body temperature is difficult. Detailed results on local reactions were not provided but a statement was made that small lumps were detectable by palpation on the day after inoculation in some animals. There is no indication of how long these persisted. That study indicates that following vaccination at the recommended dose, systemic reactions can occur, such as diarrhoea, anorexia, depression, vomiting and increase in body temperature.

In a third study, 44 x 6-7 week old SPF piglets divided into 5 groups of 6 vaccinates each plus 2 two sentinels and two groups of 2 controls each were used. This study was conducted in two phases, the first comprising Groups A, B, C and F1 and the second comprising groups D, E and F2. The study was designed to demonstrate that antigen produced in fermenters provided an equivalent level of efficacy to that produced by monolayer culture and that production in fermenters also provided a consistent antigen. The data do, however, also provide some evidence that safety of material produced in fermenters is comparable to that manufactured on monolayers.

During the first phase of the study no adverse reactions to vaccination were recorded but detailed records were not collected. There is no indication of whether or not adverse reactions following vaccination were seen during the second phase. However, a statement is made that no clinical signs of CSF were recorded. No significant increase in body temperature was recorded although again some animals showed a slight temperature increase following vaccination. None of the animals showed increase in body temperature > 40.5 °C. Rectal temperatures were recorded before vaccination but the time of recording is not specified.

This study only shows that vaccine from a batch produced in fermenters has slight effects on body temperature. There was a lack of information on local and histological reactions.

In a fourth study, a single vaccine dose of antigen (32 µg) was administered into a 4 ml volume. This occurred because in these studies early batches of vaccine were used which were formulated to contain 8 µg of antigen per ml. Consequently, in these studies a double dose of adjuvant was applied. Since the adjuvant is mineral oil it may be anticipated that local reaction will primarily be due to this component and the data are therefore useful in assessing the possible adverse effects of vaccination. Twenty 6-7 week old SPF piglets were divided into 2 groups of 6 vaccinates (dose: 32 µg) and two groups of 2 controls. Control animals received no placebo. Two additional sentinel animals were included in each group of vaccinates. Vaccinated animals were observed following vaccination and although detailed records are not provided the study report indicates that no adverse reactions were recorded. None of the animals showed severe increase in body temperature. Slight and transient temperature elevation was recorded following vaccination in some pigs. Local reactions were not recorded.

In a fifth study the vaccines used were formulated to contain 8 µg of E2 antigen per ml and therefore doses of 4 ml were inoculated. Twenty SPF pigs aged 6-7 weeks divided into two groups (A and B) of 6 vaccinates plus 2 sentinels per group, and two groups of 2 unvaccinated controls each. The trial report indicates only that no abnormalities were detected following vaccination but no details are provided and temperatures during the immediate post vaccination period were not recorded. Following challenge (at 3 months post vaccination) macroscopic and histological examination of the injection sites of the animals in group A was performed. The data relating to the vaccinated animals indicate that significant local reaction had occurred and persisted. In some cases this had led to abscess formation and in all cases the size of the reaction and particularly the depth of the reaction was important. Further to microscopic observations, it appears that all lesions consisted of an area of fibrous tissue containing vacuoles that were optically empty but have presumably contained oil or fat. The vacuoles were surrounded by a demarcation zone with some cell-debris, lymphocytes and macrophages. Some of the central spaces were completely filled with purulent material and can be described as an abscess. When the fibrous tissue was located in muscle tissue, the muscle fibers had often become necrotic or had disappeared. That study provides data on macroscopic and microscopic reactions following the administration of the vaccine at the recommended dose (32 µg) but using twice the amount of adjuvant.

According to the importance of the disease, the benefits of the vaccination should outweigh the risk and such lesions are therefore acceptable. However, the nature of the lesions should be clearly mentioned in the SPC. The Committee regrets the absence of local examination the days following vaccination. Ideally, the company should have provided data on macroscopic and microscopic reactions using the recommended dose of antigen and the recommended amount of adjuvant. Further studies were provided by the applicant and are discussed under section C2.

Another study was designed primarily to provide preliminary data on vaccine stability and efficacy following storage. Thirty-two SPF pigs aged 6-7 weeks divided into three groups of 8 vaccinates plus 2 sentinels per group, and one group of 2 unvaccinated controls. Detailed records of observations for systemic reactions were not provided but a statement indicating that two of the pigs in group A vomited regularly and showed retarded growth was provided. No other adverse effect was observed. There is no indication of whether local reactions were apparent.

In another study, 50 SPF pigs aged 6-7 weeks were divided into six groups of 6 vaccinates plus 2 sentinels per group, and one group of 2 unvaccinated controls. Except for the sentinels, pigs were challenged 3 weeks after vaccination. No systemic reaction was observed. No significant increase in body temperature was seen. Two animals in group A, which received the lowest dose of antigen (1 µg), contained in 2.0 ml, showed a temperature elevation peaking at 40.2 °C and 40.8 °C but this did not seem to be related to the effects of vaccination directly. As observed in other studies, some animals which received larger doses of antigen showed a mild and transient temperature

elevation. No other effects were observed. As in most of the previous studies, hyperthermia is difficult to evaluate since rectal temperatures were not recorded before vaccination. The increase of body temperature is not obvious in pigs vaccinated with the higher doses. Three weeks after challenge (6 weeks after vaccination), the injection sites of two animals from each vaccinate group were excised and examined macroscopically. With the exception of one animal in group F (32 µg in a volume of 4 ml) which showed no abnormalities all exhibited local granulomas with abscesses. Reactions varied in size from 10x20x30 mm up to 50x20x60 mm. The similar nature of these reactions, despite the variation in antigen dose provides a clear indication that these reactions are due to the adjuvant rather than the antigen.

In the last study 68 conventional pigs (from CSF free farm) were divided into two groups of 30 vaccinates and one group of 8 placebo controls. Group A was vaccinated once only and Group B twice. Comments were made on results obtained in group A (once vaccinated) and group C (placebo). None of the pigs showed general clinical signs. None of the vaccinated animals showed any significant increase in body temperature following the first inoculation. One pig of group A showed a slight transient swelling on day 1. Examination of the injection sites at slaughter showed no detectable reactions amongst the controls at any interval. However, amongst the vaccinates a large proportion exhibited detectable reactions at injection sites. Proportion of injection sites with reactions were 9/10 in Group A1 (slaughtered at 15 weeks of age), 6/10 in Group A2 (slaughtered at 19 weeks of age) and 7/10 in group A3 (slaughtered at 23 weeks of age). These reactions were mainly small jelly-like tumorifactions with small granulomas and occasional abscesses. These results indicate that in most animals reactions are still detectable up to 13 weeks after vaccination. The nature of the reactions does not seem to change over time and the absence of reactions at some sites probably reflects an initial lack of reaction rather than indicating that reactions had resolved. That study provides individual data on rectal temperatures, general and local reactions and macroscopic observations after the administration of the vaccine at the recommended dose and volume, in 10 weeks old piglets.

In conclusion, these studies show that the administration of the vaccine can induce systemic reactions as vomiting, diarrhoea, anorexia, depression and transient increase in body temperature. Local reactions as transient swelling has been recorded in one pig, but there is an important lack of information on this part. Macroscopic examinations have shown that in many cases, abscesses will develop and persist at the injection site up to 13 weeks after vaccination.

C.2. Safety of an administration of an overdose.

An overdose study is presented which examines overdose of antigen in three separate batches of vaccine. The vaccine batches used in these studies were manufactured in 50 litre fermenters and formulated according to the final manufacturing procedure. Nine 7 week old SPF piglets were divided into three groups of three animals. They were all vaccinated twice with a dose of 64 µg on day 0 and 32 µg on day 14. (The results after the second vaccination are presented in the next section C.3.). Careful clinical examination of each animal was conducted daily for 15 days before the first inoculation and for 30 days thereafter. Injection sites were examined and palpated daily for 19 days after the first injection and rectal temperatures were recorded for 7 days prior to vaccination and for 18 days thereafter. At no time throughout the study were any adverse general reactions recorded in any of the animals. Feed intake and general behaviour were unaffected by vaccination. A slight increase of rectal temperature has been recorded the day following the vaccination. At no time throughout the study were adverse local reactions recorded. On palpation no swellings were observed and none of the animals displayed signs of pain. The safety of the administration of an overdose for 7 week old piglets is acceptable.

Two additional studies were submitted in response to the List of Questions.

In the first one, 17 piglets of the minimum age of administration divided into two groups (C, E; with and without maternal antibodies against E2) were vaccinated four times in the neck with the E2 vaccine using

at the first inoculation twice of the recommended dose (64 µg E2 in 4 ml double-oil-emulsion, DOE) and following the recommended dose (32 µg E2 in 2ml DOE). Simultaneously two groups with 9 piglets each (D, F; with and without maternal antibodies) were treated in the same way with sterile PBS. The application scheme was identical for all animals: First vaccination at 2 weeks of age at the left upper side of the neck, the second vaccination at 6 weeks of age in the lower region of the same side, the third vaccination at 8 weeks of age at the upper right side and the fourth vaccination at the lower right side of the neck at 10 weeks of age. All pigs were sacrificed 2 weeks after the last vaccination. All piglets tolerated the vaccinations well. None of the piglets developed fever (rectal temperature > 40.5 °C) for more than one day after the vaccination. Four hours after vaccination the rectal temperature increased (mean value) from 39.1°C to 40.1°C and came back to 39.6°C the next day. Slightly elevated rectal temperatures (40.0 to 40.5 °C) were only observed in three piglets on single days. The macroscopic and microscopic investigations were therefore performed 2, 4, 6 and 10 weeks after vaccination. At 2-4 weeks after vaccination (right side of the neck), minor local reactions and moderate local reactions were observed. Swellings of 20 - 40 mm have been observed up to 2-3 weeks after the overdose. Granulomatous lesions with microabscesses were most prominent, but an occasional larger abscess was observed in three pigs in both groups. In some cases no reactions were found. At 6 to 10 weeks after vaccination (left side of the neck), small changes were visible remnants of the vaccination. The results give the overall impression that the maternally immune pigs showed slightly more alterations than the pigs without maternal antibodies against E2.

In the second study, six adult multiparous sows were vaccinated twice, once at each side of the neck, first twice the recommended dose (64µg E2 in 4 ml DOE) and second the recommended dose (32µg E2 in 2 ml DOE) was used. The interval between both applications was 30 days. Simultaneously a second group was treated the same way with sterile Phosphate buffered saline (PBS). All sows were sacrificed 40 days after the last application. The sows were observed daily for general and clinical symptoms at least 2 days before the first inoculation and continuously until day 14 following each vaccination. The injection sites were inspected and palpated for local reactions daily, also the rectal temperatures were measured daily. The sows were kept through farrowing and at the end of the trial after weaning of the piglets they were euthanised and the injection sites were investigated macroscopically and microscopically. All sows tolerated both vaccinations well. None of the sows developed fever (> 40.5 °C) or elevated temperature (>40.0 °C) which could be related to the vaccination. Only one pig showed an increase of rectal temperature (40.9 °C) for one day (day 10) after the second vaccination. This increase was considered not to be related to the vaccine but rather the start of parturition at the same day. The local findings were limited to swellings from 1 to 3 mm thickness which in both groups were observed for a longer period after the second vaccination. The swellings were not painful. Reduced appetite was observed after the first vaccination in one animal. After the second vaccination 5 of 6 sows showed at different time points reduced appetite, whereas in two cases this sign was observed around the day of parturition. In three cases reduced appetite was observed only 20, 24 or 36 days after the second vaccination and was considered not to be related to the vaccine. In the placebo group reduced appetite was only observed in one animal on day 32 after the application. All sows farrowed without complications. The mean number of piglets born alive in both groups was 11 (s.d. Group A = 2.6, Group B = 2.7), the number of dead born piglets was 7 in the vaccine group and 15 in the placebo group. The macroscopical and microscopical investigations of the injection sites were therefore performed 40 (left side) and 70 days (right side) after the vaccination. Forty and 70 days after vaccination, small changes are visible remnants of the vaccination. Most lesions are small and when not intramuscular, or with abscesses, would pass consumer tests. It is noticeable that granuloma and microabscesses were also found in the PBS treated placebo group. Because the neck is the site of choice for intramuscular administration of any medication in the pig, it has to be assumed that these alterations were caused by previous treatments or vaccinations and therefore not all findings in the vaccine group were caused by the E2 vaccine. It can further be concluded that the E2 vaccine is as tolerable as other products routinely used for treatment of sows.

These 2 studies have been performed according to the Note for Guidance on the use of adjuvanted veterinary vaccines. This guideline recommends that vaccines that produce abscesses are unlikely to be

acceptable. Independently of the time post vaccination, abscesses or micro-abscesses have been observed at 42% of the injection sites in the piglets and half of the injection sites in the sows (probably overestimated in the latter). The frequency of occurrence of this adverse reaction is rather high. However, the Applicant has provided a risk/benefit analysis from which it can be concluded that the benefits exceed the risk.

Further to the assessment of these studies, it has been mentioned in the SPC under the section “overdose” that the administration of an overdose of the product can result in a moderate swelling at the injection site that can persist for 2 – 3 weeks.

C.3. Safety of the repeated administration of one dose.

In the first study presented in this section, 68 conventional pigs were divided into two groups of 30 vaccinates and one group of 8 placebo controls. Group A was vaccinated once only and results were presented in the part “Safety of the administration of one dose”. The results for group B (vaccinated twice) and group C (placebo) will only be considered here. None of the pigs showed general clinical signs. Their general appearance and their food intake were normal throughout the observation period. However, there are no data on growth performances. Body weight was not measured. None of the vaccinated animals showed significant increase in body temperature recordings during the critical initial 7-day period following the first inoculation. In contrast, following the second inoculation, 21/30 vaccinates in Group B showed a temperature increase of $> 0.5^{\circ}\text{C}$ on the day after injection. By the second day the temperature of many animals had returned to normal but the results show that a small mean increase was still detectable. None of the animals showed detectable local reactions throughout the 13-day observation period. Examination of the injection sites at slaughter showed no detectable reactions amongst the controls at any interval. However, amongst the vaccinates a large proportion exhibited detectable reactions at one or both injection sites. These reactions were mainly small jelly-like tumorifications with small granulomas and occasional abscesses. In most animals, reactions were still detectable up to 16 weeks after vaccination. The nature of the reactions does not seem to change over time and the absence of reactions at some sites probably reflects an initial lack of reaction rather than indicating that reactions had resolved.

The second study also described above in section C2, where nine 7 week old SPF piglets were divided into three groups of three animals, provides useful evidence in respect of the safety of repeated administration. However, the second dose was given only 14 days after the first. At no time throughout the study were adverse general reactions recorded in any of the animals. Feed intake and general behaviour were unaffected by vaccination. No significant increase in body temperature was recorded in any animal during this study. Temperatures fluctuated during the course of the study but remained within normal limits at all times. It is especially noticeable that there was no tendency for increase in body temperature to occur following the second inoculation contrary to what had been observed in some other studies. No significant increase in body temperature was observed following the second dose. At no time throughout the study were adverse local reactions recorded in any of the animals. Even on palpation no swellings were observed and none of the animals displayed any signs of pain. That study shows lower reactions than the previous study. However, the scheme of administration did not conform to that recommended by the Applicant.

The third study was performed on 20 conventional landrace pigs aged approximately 8 months divided in 4 groups. Groups B and C were vaccinated at 3 months of age with an amount of 16 and 4 μg E2 respectively in 2 ml adjuvant, and that pigs in group D received at 3 months a placebo 2 ml of SF900 medium. At no time throughout the study were adverse general reactions recorded in any of the animals. Food intake was reported to be normal. However, there are no data on growth performances. Body weight was not measured. Following the first inoculation there was a very slight elevation of rectal temperature amongst the vaccinates which lasted for one day only. This change was not recorded amongst the placebo treated animals. Following the second inoculation at day 28, the temperature elevation in some animals

was maintained for two days. The peak temperature recorded was 40.2°C. In that study, increases in body temperature were observed after the administration of one dose and after repeated administration. This inconsistent effect, as compared with other studies, could be ascribed to the double amount of adjuvant used. Following inoculation at day 0, two pigs each in groups B and C displayed slight local reactivity at the injection site. In all these animals a slight swelling was detectable on one day only (days 3-5) and in one animal this was associated with a slight reddening of the skin. However, none of the animals displayed signs of pain or inflammation on palpation. Following the second inoculation on day 28 no local reactions could be detected in vaccinates or placebo treated animals. Twenty one days after the second inoculation, all the animals were slaughtered and injection sites were examined. All except one vaccinate showed tissue abnormalities at the site of one or both injections. A total of 18 of the 28 sites examined (on the vaccinates) showed abscesses which were mainly located in the connective tissue and a number of sites showed other less severe abnormalities. In contrast, only one injection site on one placebo treated animal showed abnormality and this was a subcutaneous abscess.

These studies show that the repeated administration of the vaccine in piglets and in finishing pigs can induce an increase in the rectal temperature following the second injection. No other signs of systemic reactions are observed. Slight local reactivity at the injection site can be recorded, but it does not seem to be related to repeated administration. As in other studies, macroscopic examinations show that in most animals granulomatous reactions and abscesses are detectable that will persist until slaughter.

The safety of the repeated administration of the vaccine in pregnant sows and in animals of the minimum age of vaccination was further assessed. These studies already described under section C.2. showed that the repeated application of the vaccine was well tolerated in pregnant sows. Two week-old piglets were more sensitive as shown by the higher occurrence of swelling at the injection site which disappeared within approximately one week after vaccination.

C.4. Examination of reproductive performance

Two studies which are reported under section C.8. of Part 4 are also provided in this section. For both studies, detailed results of clinical and local reactions after vaccination are not provided. No unexpected reactions were observed. However, the sows were moved from Germany to high-containment facilities of ID-DLO after vaccination 2 weeks before challenge and therefore no information on local and systemic reactions is available. The company has provided further argumentation to support that the apparent low fertility rate is independent from vaccination. In the second study, 27 sows were moved in the sixth week of gestation from Germany. All sows were vaccinated 24 days before insemination and 9/27 sows were once more vaccinated 17 days after insemination. There is a lack of information on the rate of fertility in vaccinated sows and also on systemic/local reactions.

No information is available on the possible effect of vaccination on lactation. Since a mild and transient increase in body temperature is seen in some animals, it is possible that a slight reduction in milk yield might be apparent for a few days after vaccination. Even if this did occur it is unlikely to be of any consequence for either the sows or piglets. It should be noted that in the field study growth performance of piglets born to vaccinated sows was monitored and compared with that of piglets born to unvaccinated sows. A very slight difference in weight gain was recorded but this was not significant and all the animals thrived well. There is therefore indirect evidence that lactation is unaffected by vaccination.

The vaccine is indicated for use in all categories of pig. Therefore, the possible effect of vaccination on spermatogenesis should be considered. The vaccine has been administered to young male pigs but there have been no studies in older males. The risk of untoward reaction is so slight that the best way of addressing this issue would be by post-marketing surveillance.

C.5. Examination of immunological functions

No studies to examine the potential influence of vaccination on immunological functions have been presented. The antigen contained within this vaccine is a glycoprotein and would not be expected to exert any potentiating or suppressive effect on the immune response to other antigens. The components of the adjuvant system are in regular use in other vaccines and would similarly not be expected to exert any influence. Although no data are presented there is no reason to limit the application of this product relative to the use of other vaccines.

A statement in the SPC specifies that the use of immunosuppressive drugs (e.g. corticosteroids) within 7 days before and 7 days after application of Bayovac CSF E2 should be avoided since it may interfere with the induction of immunity.

C.6. Special requirements for live vaccines

Since Bayovac CSF E2 Vaccine does not contain any live agent these requirements do not apply. It is, however, worth noting that the antigen is treated with binary ethylene-imine during production. This treatment will destroy nucleic acid. This is important because the vaccine is produced in insect cells using a baculovirus vector. Current knowledge of these cells and of baculoviruses themselves is such that we cannot be certain that these materials are free of all possible agents which might, in unusual circumstances be transmissible to other species either directly or by recombination or reassortment. However, as a consequence of this treatment no such transmission is possible from this vaccine.

C.7. Study of residues

Composition of the Bayovac CSF E2 per dose (2 ml)

All of the components Marcol 52, Montanide 80 and Montanox 80 are listed in Annex II of Council Regulation 2377/90/EEC and thus are not subject to maximum residue limit determination.

The preservative, thiomersal is also included in Annex II for use in veterinary vaccines at concentrations up to 0.02%. Since the concentration of thiomersal in Bayovac CSF E2 vaccine is 0.0035% this component is also not subject to a maximum residue limit determination.

The E2 antigen itself is a glycoprotein and will therefore be metabolised in the host with the constituent amino acids either being processed or scavenged for protein synthesis.

The culture medium for insect cells consists of a mixture of inorganic salts and amino acids, which will also be metabolised by the target animals.

The phosphate buffered saline used as diluent for the active ingredient is pharmacologically inert and will contribute to the sodium, potassium, chloride and phosphate pools within the body.

Consequently, residues following use of this vaccine will not be an issue and the Applicant's proposal for a nil withdrawal period is appropriate.

C.8. Interactions

No data were provided by the Applicant. However, the company has recommended to avoid the use of immunosuppressive veterinary medicinal products (e.g. corticosteroids) or modified live PRRS vaccines within 7 days before and 7 days after application of the vaccine.

D. FIELD STUDIES

The applicant has conducted a limited field study on one farm in which a large number of animals of various categories were used and in which both single and double vaccination was employed. The study included very young piglets both with and without maternal antibodies and is reported in Part 4 section D.

A total of 200 fattening pigs, 75 sows and gilts and 120 piglets were included in the trial. Of the fattening pigs, 150 were vaccinated and 50 received a placebo. Of the sows and gilts, in all reproductive stages, 25 were vaccinated once and another 25 twice, 6 weeks apart. Further 25 comparative sows and gilts were inoculated twice with placebo. A total of 60 piglets from placebo treated sows were divided in three groups of 20 piglets each. Additional 60 piglets with maternal antibodies from twice vaccinated sows were also divided in 3 groups of 20 piglets each. All pigs vaccinated and placebo treated were monitored for general and local signs. Specified groups of 20 vaccinates and 10 controls, each of the fattening pigs, the sows or gilts as well as the piglets treated twice were submitted to detailed monitoring, including measurements of rectal temperature and palpation of injection sites, and their body weight was monitored. The observation period lasted from 2 days before to 14 days after each inoculation. All included sows and gilts were monitored for their reproductive data (course of pregnancy, parturition, litter size, weaned piglets) following the treatments.

None of the animals showed any clinical signs following vaccination. Food intake and general condition remained unchanged in all animals. All 150 vaccinated fattening pigs tolerated the inoculation well without local reactions, as did the 50 placebo treated animals. None of the closely monitored 20 vaccinates and 10 controls showed swelling on palpation. Only 12 of 50 vaccinated sows and gilts showed a slight swelling at the injection site, which generally receded within 1 to 3 days. After revaccination slight transient swelling receding also generally within 1 to 3 days was palpated in 12 sows and gilts. No swelling could be detected in piglets. It is particularly noticeable that in this study, following first vaccination, there was no obvious evidence of even mild increase in body temperature comparable to what had been recorded in other studies. Even following the second vaccination only a very slight increase in temperature was noted and this was recorded in both the vaccinate and placebo groups. The individual reproductive performance data for the animals is provided within the report but the overall performance of the groups indicates that vaccination with either one or two doses had no impact on reproductive performance. In both gilts and sows initial vaccination took place either before insemination or during pregnancy with some animals in each trimester at the time of vaccination. Growth performance was assessed within this trial by recording of weight gain in representative samples of the fattening pigs and the piglets. In fattening pigs the mean increase in body weight during the 14 days following inoculation was compared with that of the placebo treated animals. Both groups gained weight satisfactorily and no significant difference could be detected. However, the number of pigs included for performance measurements can be considered as low. In piglets weights were recorded over a more extended period providing a more rigorous assessment of the potential impact of vaccination. In animals both with and without maternal antibody weight gain was slightly reduced in the vaccinates. The differences were, however, small and not statistically significant.

E. ECOTOXICITY

The assessment of the potential environmental risk derived from the use of Bayovac CSF E2 was performed according to the EMEA Guidance EMEA/CVMP/074/95.

Bayovac CSF E2 does not contain live organisms. The host range of the baculovirus is almost exclusively restricted to insects. A transmission to non-insect animals is not possible because baculovirus nucleocapsids are unable to uncoat in the majority of mammalian cells, the notable exception being

hepatic cell lines. Although the DNA uncoats in these cells, the baculoviral promoters remain quiescent due to a lack of required transcription factors and virus replication does not occur.

Shedding of live organisms does not occur, as Bayovac CSF E2 is an inactivated subunit vaccine and does not contain live organism.

After harvest of the E2 antigen, the genomic DNA of BacE2[-] virus is chemically destroyed during the production process of the product. Successful baculovirus inactivation is checked by plaque titration, where the requirements are $< 1 \text{ virus}/10\text{m}^3$ of inactivated fluids. A transmission of genomic characteristics can therefore be denied.

For the E2 glycoprotein, the ingredients of the used in-organic buffers and the culture media toxic effects can be denied. Thiomersal was included in Annex II of the Commission Regulation 2377/90 and can be used in vaccines in quantities up to 0.02%. The use of Montanide, Montanox (Polysorbate) and Marcol (Paraffinum liquidum) were also evaluated and published without restrictions in Annex II.

No toxic effect of excreted metabolites are expected. The E2 glycoprotein, the ingredients in-organic buffers and the culture media will be metabolised by the animal similar to substances of animal origin and can therefore not be differentiated. The oil ingredients of the adjuvants will not be metabolized in a relevant amount and will be excreted over a long period in small quantities. The use of thiomersal in veterinary medicinal products is common and therefore was evaluated according to Commission Regulation (EEC) 2377/90 and published in Annex II of the regulation. Toxic effects cannot be expected with the quantities used in the product.

The possibility of exposure and the likelihood of hazards occurring by use of Bayovac CSF E2 can be classified as "negligible". No specific risk management is necessary.

In conclusion, there is no risk for the environment associated with the use of this vaccine.

4. OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY TRIALS

A. INTRODUCTION

The structure of CSF virus has been extensively studied and it is well established that the E2 glycoprotein alone can provide protection against infection. However, all naturally infected animals develop antibodies against a range of other antigens as well as E2 and it is therefore possible to distinguish between such animals and those vaccinated with this product provided an appropriate diagnostic test is available.

B. GENERAL REQUIREMENTS

It should be noted that early studies were conducted using vaccine prepared on a pilot scale. In particular, early batches were prepared by monolayer culture whereas the production method and the method used in some later studies, relies on production in suspension culture. In general, however, the studies conducted using monolayer derived material may be regarded as fully applicable to the final product.

Data were required from laboratory and field trials on:

- **Each category of each target species**
The target species is the pig. Laboratory efficacy studies have been performed in 2 week old pigs, in 6-7 week old pigs, in 10 week old pigs and in pregnant sows and gilts.
- **Each recommended route of administration**
All pigs have been vaccinated using the recommended route of administration.
- **Proposed schedule of administration**
Challenges have been performed in 6-7 week old pigs, in 10 week old pigs and in pregnant sows and gilts administered one dose of vaccine. Challenges have been performed in 2 week old pigs administered twice the vaccine at 6 weeks apart and in pregnant sows and gilts administered twice the vaccine at 5.5 and 6 weeks apart.
- **Effect of passively acquired and maternally derived antibodies**
Piglets vaccinated as young as 2 weeks of age have been challenged at 6 months of age
- **Claims regarding onset and duration of protection**
Studies on onset and duration of immunity have been performed in 6-7 week old piglets.

A statement on GLP and GCP is provided. No studies have been submitted concerning the excretion of challenge virus in vaccinated boars. There is no indication that boars react differently from piglets, fattening pigs or pregnant sows, with regard to the induction of immunity following vaccination. It is assumed that vaccination also will lead to a drastic reduction or even prevention of virus via the semen.

C. LABORATORY TRIALS

C.1 Establishment of a Challenge Model.

An intranasal challenge model has been used throughout the efficacy studies. The intranasal route of inoculation is the normal route of transmission of the disease. Intranasal administration seems more appropriate than parenteral administration as the parenteral challenge would bypass mucosal and possibly some local cellular immune mechanisms. Furthermore, in vaccinated animals administration by a parenteral route may result in partial neutralisation of the challenge virus due to exposure to circulating antibody.

In the challenge model used in the presented studies, death of unprotected animals was routinely recorded at periods of between 10 and 17 days post challenge. This is entirely consistent with the course of natural

infection and the technique therefore provides a sound model upon which efficacy can be judged. In principle, the challenge is a heparinised blood sample from an infected pig which has been titrated in swine kidney cells. The challenge inoculum is prepared in a standard way by dilution in PBS so that the challenge dose is consistent between studies. Two different challenge strains have been used. In the majority of studies, the highly virulent *Brescia* strain has been used. For studies specifically designed to examine transplacental transmission the *Zoelen* strain was selected. This latter strain is described in the dossier as being less virulent but this description is rather misleading. The *Zoelen* strain does result in much less severe clinical signs but the important point is that it permits the survival of the infected animal thus permitting transmission across the placenta. It is thus of different rather than lower virulence and the strains selected for challenge are both well suited to the purposes of the studies in which they have been used.

The vaccine does not inhibit the development of anti-E^{RNS} antibodies in pigs challenged with strains of low and mid virulence.

C.2 Determination of the Vaccine Dose.

It is worth noting that the requirement for live CSF vaccines, as described in Ph. Eur. monograph 0065, is that each dose of vaccine should contain 100 50% protective doses (PD₅₀). This requirement is clearly not appropriate to this subunit vaccine and the purpose of the studies to determine the vaccine dose was essentially to establish the 95% protective dose (PD₉₅). Each dose of vaccine is designed to contain one PD₉₅. Therefore, the final vaccine should protect 95% of pigs against disease.

Two studies specifically designed to determine the vaccine dose are reported.

In the first study, 26 x 6-7 week old SPF piglets were divided into 4 groups of 6 vaccinates and one group of 2 controls. The piglets were vaccinated intramuscularly in the neck with 0, 0.25, 1, 4 and 16 µg vaccine doses. At day 28 post-vaccination, they were challenged intranasally with 100 LD₅₀ CSFV Strain *Brescia*. The serological response to the vaccinations was monitored with an ELISA test at day 0, 14, 21, 28 post vaccination and at death (day 28 post challenge). Systemic examination was performed daily for 22 days post challenge, and body temperature recorded daily 14 days post challenge. At time of death or on day 22 when remaining animals were slaughtered tissue samples were collected and examined for CSF virus. Unvaccinated control animals developed signs of CSF 3 days after challenge: fever, huddling, loss of appetite, paresis posterior and depression were seen and worsened till death. All the vaccinated animals, even those receiving the largest vaccine dose showed clinical signs of disease in addition to increase in body temperature. Peak temperatures occurred in all animals between day 3 and day 6 post-challenge. Animals vaccinated with 16 µg of antigen all survived, but one showed clear evidence of tissue infection. The minimum detectable titre of virus in the re-isolation studies was 1.05 log₁₀ TCID₅₀/ml and therefore significant infection may have been undetected. (It should be noted that according to the information provided in respect of the challenge inoculum 100 LD₅₀ is provided by 10^{3.09} TCID₅₀). Nevertheless, the study clearly demonstrates dose regression and it is obvious that the highest vaccine dose is beneficial. Seroconversion was defined as a titre > 25 in the virus neutralisation test. Vaccination elicited a response in all groups except the group administered the lowest vaccine dose and it is particularly noticeable that the priming effect of vaccination was dose dependent. All but one animal from the group vaccinated with the highest dose had seroconverted on day 28 after vaccination. Four out of six animals from the group vaccinated at 4 µg and 2/6 from group vaccinated at 1 µg seroconverted on day 28 post-vaccination. It is clear that none of the animals were completely protected against infection but that those given the highest vaccine dose were better able to overcome the infection. On the basis of these data the Applicant has calculated the dose which protects 50% of the vaccinates (PD₅₀). PD₅₀ is 2.5 µg and by extrapolation the PD₉₅ is 28.35 µg. These figures are correct if one regards absence of detectable antigen or viraemia as evidence of protection but not if one considers protection against disease signs. Overall it was clear from this preliminary experiment that higher vaccine doses needed to be investigated.

In the second study, 26 x 6-7 week old SPF piglets divided into 3 groups of 6 vaccinates and one group of 2 controls were used. Two additional sentinel animals were included in each group of vaccinates. Control animals were vaccinated with 2ml of adjuvant only but the sentinels remained unvaccinated. Vaccine doses were 0, 2, 8, and 32 µg. This study was conducted in a similar manner to the previous study and used vaccines prepared in a similar way (i.e. from monolayer grown culture) but from a different bulk antigen. Challenge was carried out 21 days after vaccination compared to 28 days in the previous study and, as noted above, two unvaccinated sentinel animals, which were not challenged were included in each vaccinate group. Observations and samples were carried out as before. Animals vaccinated with either 2 or 8 µg of antigen were not fully protected against challenge. All of these animals exhibited increase in body temperature and some showed both leucopaenia and thrombocytopaenia. Furthermore, one animal in each group exhibited viral antigen in the tissues at slaughter and sentinels in contact with both groups clearly became infected. In contrast, none of the animals vaccinated with 32 µg of antigen showed any evidence of haematological disturbances and, with the exception of one animal on one day no increase in body temperature was observed. Most importantly, neither of the sentinel animals in contact with this group showed any evidence of infection. Virus neutralisation titres recorded in this study indicate a high level of antibody both 14 and 21 days after vaccination amongst the animals in the group vaccinated with the 32 µg dose with lower and more variable titres achieved in the groups which received the lower vaccine doses. All the animals in that group showed a dramatic increase in titre following challenge and although some animals in the other groups showed similar responses these were less consistent suggesting that these lower doses could not be relied upon to provide adequate immunity in all cases.

It may therefore be concluded that the choice for an antigen dose of 32 µg seems to be appropriate regarding the results of antigen detection. However, increase in body temperature was observed in one animal following challenge and viraemia was not tested. Therefore, the vaccine provides a reduction of clinical signs rather than prevention of the infection. Moreover the absence of horizontal spreading is not fully proven here as data on viraemia are lacking.

C.3. Onset of protection.

Two studies are described which specifically examine the time of onset of immunity.

In the first study, 20 x 6-7 week old SPF piglets divided into 2 groups of 6 vaccinates (vaccine dose: 32 µg of E2 antigen in a volume of 4 ml, animals vaccinated once only) and two groups of 2 controls. Control animals received no placebo. Two additional sentinel animals were included in each group of vaccinates. Animals in groups A and C1 were challenged intranasally with 100 LD₅₀ CSFV Strain Brescia at day 14. Animals in groups B and C2 were challenged intranasally with 100 LD₅₀ CSFV Strain Brescia at day 21. Body temperature was recorded daily 20 days after challenge, and systemic examination performed daily up to the end of the trial. The serological response to the vaccination was monitored with an E2 antibody specific ELISA test. Blood samples were taken on days 0, 2, 4, 7, 10 and 14 after challenge for haematology and viraemia testing purposes. Animals challenged 2 weeks after a single vaccination, whilst surviving challenge, were not fully protected. No significant leucopaenia or thrombocytopaenia was recorded in these vaccinates. In contrast, when animals vaccinated in the same way were challenged 3 weeks later there was a clear improvement in protection. Only one animal exhibited an increase in body temperature in excess of 41°C, none showed leucopaenia or thrombocytopaenia and neither virus nor antigen could be detected. Most importantly, there was no evidence of any transmission to the sentinel animals. Control animals for both groups showed typical signs of disease and were clearly heavily infected. These observations are consistent with the results of serum neutralisation assays. At two weeks post vaccination titres of all the vaccinates were low and detectable increase above the threshold level was only observed in two animals. In contrast, all animals in group B exhibited detectable titres at the time of challenge (3 weeks after vaccination). Furthermore, the increase in titre following challenge was

generally more marked in Group B suggesting that the longer interval between vaccination and challenge was beneficial in terms of the ability of the vaccinates to respond.

In this study, vaccinations have been performed using twice the amount of adjuvant (4 ml). However, it was concluded that the use of a double quantity of adjuvant compared to the recommended dose did not affect the immune response.

Since the results of this study showed that challenge at two weeks post vaccination was partially effective a further study was conducted in which challenge at both 1 and 2 weeks after vaccination was performed.

Thirty 6-7 week old SPF piglets divided into 2 groups of 10 vaccinates and one group of 10 controls. In Group A, 5 pigs were challenged 14 days after vaccination and in Group B 5 pigs were challenged 7 days later after vaccination. This study differed slightly from the previous study in that the sentinel animals included in each vaccinate group also received vaccine and additional sentinel animals were included in the control group. The study was designed so that all challenges would occur on the same day and therefore the controls served for both vaccinate groups. Observations and samples were collected in a similar manner to that described for the previous studies.

Animals challenged only 7 days after vaccination failed to survive challenge and showed marked leucopaenia and thrombocytopaenia. Furthermore the in-contact vaccinates housed with them, whilst surviving showed clear signs of disease and three of these animals exhibited viral antigen in the tissues indicating that transmission had occurred and that, despite being vaccinated they were not completely protected.

On the other hand, animals challenged 2 weeks after vaccination appeared to be protected against all but the mildest signs of disease as shown by slight increase in body temperature. The contact vaccinates housed with these challenged animals did not show any sign of infection. Unfortunately because these contact animals had themselves been vaccinated and effectively were not exposed to contact challenge until approximately 18 days after vaccination this observation does not necessarily mean that transmission from the directly challenged animals could not have occurred if the contact animals were naïve. Nevertheless, the absence of any detectable viral antigen or viraemia in these challenged animals does suggest that effective protection was achieved.

Animals challenged 2 weeks after vaccination had developed low but detectable antibody titres and had also developed the capacity to respond rapidly to challenge. In contrast, none of the animals challenged one week after vaccination showed detectable antibody at the time of challenge and all responded more slowly to challenge. More careful analysis of the individual animal data shows that 6 of the 10 vaccinates in group A had detectable titres at challenge but that all were able to overcome the challenge. The contact controls in group A, whilst showing a response to challenge did so to a lesser extent than the contact animals in group B. This might indicate that the exposure of these contacts to challenge was less when the challenge was administered 2 weeks post vaccination than when it was administered only one week later. It should also be noted that if the animals challenged two weeks after vaccination were fully protected it might be anticipated that the contacts would fail to respond to challenge. Consequently, it is difficult to say with certainty that full protection against challenge was achieved at 2 weeks. Nevertheless, the level of protection achieved was sufficient to prevent transmission and therefore cause the disease to die out in the situation where all animals are vaccinated.

In conclusion, the onset of protection of 2 weeks can be determined, based on the protection against mortality and the reduction of clinical signs of the disease and the excretion of field virus.

C.4. Demonstration of equivalence of monolayer and fermenter produced antigen.

Fortyfour 6-7 week old SPF piglets divided into 5 groups of 6 vaccinates each plus two sentinels and two groups of 2 controls each. This study was conducted in two phases, the first comprising Groups A (vaccine dose: 2 µg), B (8 µg), C (32 µg) and F1 (not vaccinated) and the second comprising groups D (32 µg), E (32µg) and F2 (not vaccinated). All animals were challenged intranasally with 100 LD₅₀ CSFV Strain *Brescia* at Day 21 post vaccination. The study was designed to demonstrate that antigen produced in fermenters provided an equivalent level of efficacy to that produced by monolayer culture and that production in fermenters also provided a consistent antigen. To this end dose regression was examined together with the efficacy of three different batches of fermenter produced vaccine derived. The study was conducted in two phases Groups A, B, C and F1 forming the first part and groups D, E and F2 the second part. However, since the pigs were SPF from the same source and the experiments were conducted at the same premises the results from the two phases may be regarded as directly comparable.

The protection afforded by vaccine doses of 2 and 8 µg antigen in this study was comparable to that seen in earlier studies using monolayer derived material. Some of the animals in these groups survived challenge and some remained free of detectable viraemia. However, as in the previous studies the sentinel animals were clearly infected indicating that these doses were insufficient to prevent transmission.

The results recorded for Group C, which received the standard dose of antigen, are difficult to interpret. One vaccinated animal in this group died following challenge and was clearly infected showing a severe increase in body temperature, viraemia and marked leucopaenia and thrombocytopaenia. Two other animals in the group exhibited increase in body temperature of <40.5°C but neither of these showed any viraemia or other recorded clinical signs and the remaining animals appeared to be completely protected. Probably as a consequence of exposure to the severely infected animal, both the sentinels developed clear signs of infection.

In contrast, animals in groups D and E, which also received the standard dose of antigen were more consistently protected against challenge. Only two animals in Group E exhibited increase in body temperature of < 40.5°C and none in either group showed other signs of disease. Furthermore, there appeared to be no transmission of infection to the sentinels in either group.

The results for groups D and E suggest that the protection afforded by the fermenter grown antigens used is similar to that recorded in previous studies using monolayer produced material. It is therefore reasonable to conclude that the use of 10 litre fermenters will not have any adverse effect on the product efficacy. In addition, because three different antigen batches were used in the preparation of the vaccines for these groups it seems that consistency of production can be maintained when the antigen is produced in these fermenters.

Unfortunately, the data do not permit the same conclusion to be drawn in respect of production in 50 litre fermenters. There is, of course, no reason to suppose that the larger size of fermenter should have any impact on antigen quality or consistency. It is most probable that the observations recorded for group C are due to the poor performance of one animal rather than any intrinsic defect in the vaccine. This view tends to be reinforced by the fact that none of the other vaccinated animals showed significant disease signs and by examination of the serological responses. As expected, the serological responses observed in the groups receiving the lower vaccine doses were smaller than those of animals administered the full dose but these animals were still able to respond well to challenge and all the survivors exhibited titres of >1600 at the time of slaughter.

One further study was conducted in which two distinct batches of vaccine, produced in 50 litre fermenters and formulated according to the final production process, were evaluated. The study was conducted in a similar manner to previous studies with vaccinated animals being challenged 21 days post inoculation and housed with sentinel animals to detect possible transmission. Eighteen 6-7 week old SPF pigs divided into

two groups of 6 vaccinates plus 2 unvaccinated sentinels and one group of two unvaccinated controls were used. The clinical results show a very effective protection against challenge: only two vaccinates in each group exhibited temperatures equal to or greater than 40.0°C and only one animal showed a temperature elevation which lasted for 2 days reaching a peak of 40.5°C. No increase in body temperature was recorded amongst the sentinels of either group. None of the vaccinates or sentinels showed any significant leucopaenia or thrombocytopaenia. In contrast both the control animals exhibited marked increase in body temperature for several days and both died as a consequence of challenge. The effectiveness of the protection against challenge in this study is also shown by the absence of detectable virus at any time after challenge or viral antigen at the time of slaughter in the vaccinates or sentinels. The responsiveness of the vaccinates is clearly demonstrated by the serological results following challenge. Titres of the vaccinates at the time of challenge were significant and comparable to those recorded in other studies. More importantly, all the vaccinates responded rapidly to challenge and at the time of slaughter, 42 days after challenge all displayed extremely high titres.

These data therefore provide solid evidence that material manufactured in 50 litre fermenters is at least as efficacious as monolayer grown material.

In a third study provided in response to the List of Questions, the “potency of the E2 subunit vaccine produced in 50 liter fermentor batches, and determinations of the PD95 dose of the 50 liter fermentor batch” was evaluated.

Five groups of 6 SPF pigs, 6-7 week old, were vaccinated once by the intramuscular route with several E2 subunit vaccine batches with different concentrations of antigen: group A: 2 ml (16 µg/ml); group B: 2 ml (16 µg/ml); group C: 2 ml (16 µg/ml); group D: 2 ml (a mixture of the 3 antigen lots formulated in one vaccine batch; 4 µg/ml); group E: 2 ml (1 µg/ml). Two non-vaccinated pigs (sentinels) were kept in contact with the vaccinated pigs of each group (A-E) to detect virus transmission after challenge. One group (F) of two pigs was vaccinated with the dummy containing only the DOE adjuvant (E2-98-Dummy3) as a negative control for vaccination. At day 21, all vaccinated animals (A-E) and control animals (F), but not the sentinels, were challenged by intranasal route with 100 LD₅₀ of the highly virulent CSFV strain *Brescia*. The control animals in group F developed typical symptoms of CSF. The pigs were killed moribund during the experiment.

In group A, none of the animals developed any clinical symptoms after vaccination. After challenge both sentinels developed fever starting 7 days post challenge. Both animals developed clinical symptoms consistent with CSF infection from day 11 onwards. None of the vaccinates showed any symptoms or fever. In group B, none of the animals developed any clinical symptoms after vaccination. After challenge fever was observed in three vaccinates and none of the sentinels. No clinical symptoms developed in any of the vaccinated animals. In group C, two vaccinated animals developed fever after vaccination as did one of the (non vaccinated) sentinels. Both sentinels developed fever on 4 days post challenge. One of the sentinels developed clinical symptoms consistent with CSF on day 8 post challenge. The other sentinel did develop signs of general malaise 15 days post challenge. Five animals in the vaccinated group developed fever on 4 days post challenge but none of the vaccinates developed any clinical signs. In group D one vaccinate and one sentinel developed fever after vaccination. After challenge one sentinel and six vaccinates developed fever. None of the animals in group D developed any CSF symptoms. In group E one of the vaccinated pigs developed fever which lasted for four days after vaccination. After challenge both the sentinels and three of the vaccinates developed fever. One sentinel developed CSF symptoms at day 4 post challenge and was killed moribund during the experiment. The other sentinel developed CSF symptoms starting at day 10 post challenge. Virus could be recovered from the leukocytes of both sentinels and one of the vaccinates. In group F none of the animals developed fever after injection with the dummy DOE. After challenge the two animals developed fever and CSF symptoms starting 3 days post challenge.

Virus could be isolated from all animals in the experiment that developed CSF symptoms (sentinels and dummy vaccinated animals) and also from one vaccinated animal in group E. Animals with CSF symptoms almost always developed leukocytopenia and thrombocytopenia. Spleens, tonsils and kidney and ileum of all animals with CSF symptoms were positive for CSF antigen by immunofluorescent staining.

From this study it can be concluded that the recommended vaccine protects against the clinical signs of the disease as it was shown with the other methods of production. However in this last study, virus spreading was observed in most of the sentinels. From the serological data it can be seen that the serological response with the vaccine produced in fermenters is comparable to that obtained using a vaccine produced from monolayer grown culture. Therefore, it can be concluded that the recommended product is equivalent to the other methods of production taking into account the clinical and serological results.

C.5 The Influence of Maternal Antibody on the Efficacy of the Vaccine.

One study was primarily designed to examine the potential impact of maternal antibody on the efficacy of the vaccine. This study also permits evaluation of the duration of immunity, allows limited examination of the effectiveness of the vaccine in very young piglets and provides a comparison of the effect of single and double vaccination on efficacy.

Thirty-six conventional piglets aged between 2 and 10 weeks at the start of the study were divided into 3 groups of 6 vaccinates plus 2 sentinels in each group and one group of 2 control animals. Challenge, clinical observations and sampling were carried out as for previous studies and surviving animals were slaughtered 26 days after challenge.

All the piglets in Group A (animals aged 2 weeks at the first vaccination) were derived from sows which had previously been vaccinated whereas the remaining piglets were born to previously unvaccinated sows. The animals in this study were derived from a field study which is considered later and information provided within the report of this study indicates that the piglets born to unvaccinated sows were seronegative whilst those born to vaccinated sows had acquired maternal antibody to a significant titre.

Although all the vaccinated animals survived challenge, transmission occurred within groups B (animals aged of 2 weeks at first vaccination) and C (animals aged 10 weeks at vaccination). Furthermore, 5/6 animals in groups A and B and all animals in group C exhibited increase in body temperature ($> 40.5^{\circ}\text{C}$). The frequency of leucopaenia and thrombocytopenia was lower within group A than in groups B and C but was not significantly different. The results indicate that protection provided by the vaccine is not adversely affected by maternal antibody at the time of vaccination. There is even some suggestion that the level of protection afforded 6 months after vaccination was slightly better amongst those animals with maternal antibody at the time of vaccination. This is an unexpected finding and since the sentinel animals in group A also possessed maternal antibody and since the frequency and extent of increase in body temperature in group A was comparable to other groups it is not clear whether maternal antibody alone could be responsible for this protection. Examination of the virus isolation and antigen detection results does not, unfortunately, help to resolve this difficulty. One of the vaccinated animals in each group showed viraemia, each for one day only and none showed viral antigen in the tissues at slaughter. Results for the sentinel animals suggest an absence of virus in the sentinels of group A possibly indicating improved prevention of transmission.

Examination of the serological results provides some evidence that the protection afforded to group A was the consequence of vaccination rather than of maternal antibody.

These data clearly demonstrate that all vaccinated animals retained detectable humoral antibody at the time of challenge and that all were able to respond anamnesticly to challenge. In contrast, none of the sentinels responded to challenge irrespective of whether or not they had received maternal antibody.

The only conclusion that can be drawn from this study is that vaccination, at the age of 2 weeks followed by a second vaccination at 8 weeks, of piglets born to previously vaccinated partially confers protection against a challenge performed at the age of 6 months. The present study is more a “duration of immunity study” than a study on the influence of maternal antibodies.

Another study was submitted later on to support this statement that in emergency situations the basic immunisation consists of one intramuscular inoculation with 1 dose (2 ml) for all pigs over 2 weeks of age, irrespective of the level of maternal antibodies. This study was planned as a combined study to investigate the efficacy of a single vaccination with the E2 vaccine in the presence of maternal antibodies and the efficacy of maternal antibodies without additional vaccination in a challenge experiment in two week old piglets. Four groups were used: Group H: 7 piglets with maternal antibodies and vaccinated at 2 weeks of age, Group G: 7 piglets with maternal antibodies, not vaccinated; Group I: 6 piglets without maternal antibodies against E2 but vaccinated at 2 weeks of age and Group J: 4 not vaccinated control pigs without maternal antibodies. All pigs were challenged intranasally at an age of 5 weeks. Twenty-four hours later 2 sentinels were added to the piglets of group H, I, J for the detection of virus transmission.

The pigs of the control group J developed 2 and 3 days after challenge fever and showed clinical signs of CSF from days 4 or 10 and died either on days 15, 16 or 20 or were killed moribund on day 22.

In Group G (+MDA; vaccinated) the pigs developed fever from day 1-2 after challenge with a maximum duration of 9 days. All piglets had normal temperatures at the end of the trial. One pig showed dullness from day 3. One sentinel developed fever from day 10 onwards, showed clinical signs of CSF from day 15 and died on day 21. The other sentinel had fever from day 16 and showed clinical signs of CSF from day 16.

The pigs of Group H (MDA+, not vaccinated) developed fever from day 1-2 after challenge with a maximum duration of 19 days. Five piglets died from CSF or were killed moribund between day 9 and 19. Two pigs were killed at the end of the trial but both had still fever. One sentinel developed fever from day 8 and died on day 19. The other sentinel had fever from the days of contact, probably due to an inflammation of the right hind leg and died on day 15.

In Group I (-MDA; vaccinated) the pigs developed fever from day 1-2 after challenge with a maximum duration of 8 days. All piglets had normal temperatures at the end of the trial. All inoculated pigs showed some dullness from day 4 for 2 days and loss of appetite on day 6. Both sentinels developed fever from day 7 onwards and dullness for 2 days from day 4 and also some loss of appetite on day 6.

E2 antibodies can be transferred via colostrum. These maternally derived antibodies, however, are not protective against mortality after challenge with a virulent CSF strain five weeks post partum.

The single vaccination with E2 vaccine induced clinical protection against CSFV infection, even in piglets with maternally derived antibodies, as none of the piglets developed severe clinical signs or died of CSF after challenge. The vaccination however could prevent viremia and virus spread to the organs, but not completely prevent transmission of virus to highly susceptible unvaccinated sentinel piglets. This phenomenon was also found in both vaccinated groups.

C.6 Duration of Immunity.

In addition to the first study discussed in the above section C.5, a further study is reported and is specifically designed to examine the duration of immunity provided by the vaccine.

Twenty SPF pigs aged 6-7 weeks divided into two groups of 6 vaccinates plus 2 sentinels per group (challenged 3 months or 6 months post vaccination respectively), and two groups of 2 controls each. Vaccinations in this study were performed using experimental vaccines formulated to contain 8 µg of E2 antigen. Consequently the dose administered was 4 ml and each animal therefore received twice the quantity of adjuvant that would be incorporated in a normal 2 ml dose of the final product.

In two animals, leucocytes counts as low as 4.8 and 5.4 ($\times 10^9$ /ml) were recorded respectively at days 7 and 10 post challenge. It seems therefore that transient leucopenia occurred. However, no viraemia was detected and protection against clinical signs and transmission has been shown. Amongst those vaccinated animals challenged 3 months after vaccination none exhibited increase in body temperature $> 40.5^\circ\text{C}$ nor showed other clinical signs of disease. The mean peak increase in body temperature amongst those vaccinated animals 6 months after vaccination was slightly greater and two animals showed increase in body temperatures $> 40.5^\circ\text{C}$ which were associated with other clinical signs (depression, anorexia, vomiting). In neither of the groups did transmission to the sentinels occur. None of the vaccinates or the sentinels in either group exhibited viraemia or viral antigen in tissues. The serological results indicate that residual titres were detectable in all the vaccinates at the time of challenge although the mean titre at 6 months (group B) was significantly lower than that at 3 months (group A). Nevertheless, all the animals in both groups responded anamnistically to challenge.

It is particularly interesting to note that one animal in each vaccinate group initially showed only a minimal serological response to vaccination but maintained (and slightly increased) the titre at the time of challenge. This suggests that the antibody generated by the vaccine is rather long lived and there is no doubt that the immune memory elicited is well preserved. It may therefore be concluded that at both 3 and 6 months after vaccination, the level of protection afforded was sufficient to prevent transmission although at 6 months post vaccination the animals may have become transiently infected. On the basis of these results, coupled with those from the previous studies, it may be concluded that effective immunity is maintained for at least six months following a single vaccination.

Since the volume administered was 4 ml and each animal therefore received twice the quantity of adjuvant, a separate trial was provided to support the extrapolation to the duration of immunity with the final product.

In that study, "Effect of maternal antibodies on vaccination as evaluated by challenge after 3 months", the efficacy of the vaccination with the routine dose (32 µg E2 in 2 ml) was demonstrated at 3 months post vaccination. For this trial two groups of piglets, one group with maternally derived antibodies and one group without, were vaccinated at the age of 2 weeks. The pigs of each of these 2 groups were separated in two subgroups. One of the subgroups each were directly challenged at 3 months post vaccination. The other subgroup served as contact sentinels to evaluate transmission of challenge virus. All vaccinated pigs survived the challenge without showing any clinical signs and without thrombocytopenia. Leukopenia was only present in 1 of 5 directly infected pigs in each of the two groups. No virus in the organs could be detected in any of these pigs. No virus shedding to the in-contact sentinels could be detected. The vaccinated in-contact sentinels remained seronegative against E^{ms}. In contrast, the directly infected non-vaccinated control pigs and their contact sentinels developed severe signs of CSF. All the control pigs developed thrombocytopenia or leukopenia. At the end of the trial two in each subgroup of the control pigs had still virus in their organs. All control pigs were positive for E^{ms}.

This demonstrates that there is no difference with regard to efficacy between the vaccine applied as a 4 ml dose with 32 µg E2 and the vaccine applied as a 2 ml dose with 32 µg E2. Evaluating all the above mentioned results it can be even assumed that the fermenter produced vaccine batches formulated with 32 µg E2 in 2 ml induce slightly higher antibody titres than the vaccine produced with antigen derived from stationary cultures (monolayer). As there is a good correlation between the serum neutralising antibody titres and the protection it can be assumed that the duration of immunity is at least the same in vaccinates inoculated with the 2 ml dose as in those inoculated with the 4ml dose. In this study, the

challenge induced clinical signs, thrombocytopenia and leucopenia in all control pigs but failed to induce mortality in some pigs (only 2/5 challenged controls and 2/5 sentinels died). The virulence of the challenge seems to be low compared to the other experiments where generally the challenge induced 100% mortality in inoculated pigs. Moreover, virus isolation showed negative results in surviving pigs. According to the Applicant “probably the virus had disappeared because of the antibody development”.

This new study demonstrates that the recommended vaccine can induce a duration of immunity of 3 months. Since there is no difference with regard to efficacy between the vaccine applied as a 4 ml dose with 32 µg E2 and the vaccine applied as a 2 ml dose with 32 µg E2 and that a duration of immunity of 6 months has been demonstrated earlier using a 4 ml dose with 32 µg E2, the proposed duration of immunity of 6 months can be accepted.

C.8. Prevention of Transplacental Transmission.

If a vaccination programme against Classical Swine Fever is to be effective, the vaccine will need to be used on a whole herd basis. Therefore, not only should the vaccine prevent transmission of the disease between animals but it must also eliminate transplacental transmission. The applicant has conducted two studies, which are specifically designed to address the ability of Bayovac CSF E2 to prevent such transmission. Significantly, the design of the challenge used in these studies was subtly different to that used in other studies. This was necessary and appropriate because highly virulent strains of CSF usually result in death of the animal. Consequently, transplacental transmission can only occur when less severe, but more chronic forms of the disease are prevalent. The challenge strain used in these studies was strain “Zoelan” and was administered intranasally in doses designed to induce infection without causing death or obvious clinical signs of disease. This was obviously extremely difficult and the exact challenge procedure was modified in the second of the two studies reported.

In the first study, ten sows were divided into one group of 4 vaccinated twice, one group of 2 vaccinated once and one control, unvaccinated group of 4. Groups originally comprised larger numbers of animals but only those which were pregnant at the conclusion of the study were included within the report. Group A was constituted of 4 sows vaccinated twice: 2 weeks before and 4 weeks after insemination; Group B was constituted of 2 sows vaccinated once 4 weeks after insemination. Challenge was performed 8 weeks post-insemination. Following challenge of the animals the sows were observed for clinical signs for a period of 28 days. At this time the animals were slaughtered and their foetuses examined for the presence of viral antigen. In addition, blood samples were collected throughout the study to determine the serological response to vaccination and challenge.

No clinical signs of disease were noted in any of the animals following challenge. None developed increase in body temperature. Some animals were reported to show a reduced food intake. Food consumption data are not provided in the study report and these are probably not relevant as it seems most probable that this was not related to the challenge. Despite the absence of clinical signs in the sows it is clear that the challenge was effective because virus was isolated and antigen detected in the foetuses of 3 of the 4 control animals. In contrast, none of the foetuses from vaccinated animals showed any evidence of virus and transmission seemed to have been totally prevented in these groups. Confirmation that genuine protection against challenge had occurred may be derived from the serological responses of the sows. These data indicate that not only did the vaccinated animals have significant titres at the time of challenge but that they responded anamnesticly to challenge.

Examination of the individual serological results for the controls is especially interesting because there does seem to be some correlation between the titres achieved and the level of protection of the foetuses. It is clearly dangerous to draw conclusions from such small numbers but it does seem that the titre required to achieve protection against placental transmission may be rather higher than that required to prevent transmission between adults. On the other hand this may be totally misleading because there is some

suggestion that these titres were due to antibody directed against another Pestivirus, possibly BVD. Nowadays, antibodies due to BVD virus infection can be differentiated from antibodies induced by CSF virus infection, based on an NS3 ELISA. This test was not available when the trial was performed. However, for the outcome of that trial in vaccinated pigs these low level of unspecific antibodies do not play a role, as the vaccine induced antibodies are much higher than the possibly present unspecific antibodies. As the strain used in vertical transmission is different from the strain used for horizontal transmission, no conclusion can be drawn on the comparison on the amount of neutralizing antibodies providing protection.

Since the numbers of pregnant animals in the previous study was small a further study was initiated. This second study differed slightly in the timing of the vaccinations relative to insemination, the inoculum volume used for challenge, the time of slaughter post challenge and the samples collected for evaluation.

Twenty seven sows divided into three groups of nine animals each. Group A remained unvaccinated, Group B was vaccinated twice 24 days and 17 days before insemination, and group C was vaccinated only once 24 days before insemination. Challenge occurs 7 weeks after vaccination. As in the previous study none of the animals showed any clinical signs of disease following challenge but virus was isolated from foetuses of all of the control animals and antigen was also detected in the kidneys of these foetuses. None of the sows (vaccinates or controls) exhibited viral antigen in tonsil, spleen, kidney or ileum at slaughter. Some foetuses in all groups were mummified. Since mummification is not a typical sign of CSF infection these foetuses have been excluded from the summary provided in the following table. All the sows in the control group transmitted virus to at least some foetuses. In contrast, amongst the vaccinates only one animal given a single vaccination did so. Data suggest that administration of a single vaccination may not always be sufficient to protect against transmission in pregnant animals. It is immediately apparent from serological data that the antibody titre of Group B was much higher than that of Group C at the time of challenge. More importantly, although an anamnestic response was recorded in all the animals in group C, this was delayed compared to group B in all except one animal. Interestingly even in the sow which transmitted virus to its foetuses a clear anamnestic response was observed.

Taken together the data from these two studies indicate that effective protection against placental transmission can be achieved by use of the vaccine provided that two doses of vaccine are administered. In the studies described the first dose of vaccine was given prior to insemination. Consequently, it is not clear that complete protection would be achieved if the first dose was administered after insemination. However, the timing of vaccination relative to insemination is probably not as important as the rate of onset of immunity when measured in terms of placental transmission. The available data indicate that effective immunity is generated by 3-4 weeks following the second vaccination. In the majority of animals, similar protection is seen following a single vaccination dose but the occurrence of transmission in one sow is significant because infected foetuses are likely to become persistently infected and therefore act as a reservoir of disease. Furthermore, because two doses are required to achieve complete protection the use of the vaccine in an emergency situation might not be fully effective for all animals. In reality this may not be significant because the risk of transmission would certainly be reduced.

The extent of protection against challenge in late pregnancy has not been evaluated.

C.9 Additional studies

Two further studies are reported both of which are primarily intended to demonstrate the stability of the product. In both of these studies three different batches of pilot scale vaccine, produced by monolayer growth, have been examined for efficacy after storage for 12 or 21 months.

In the first study, 32 SPF pigs aged 6-7 weeks divided into three groups of 8 vaccinates plus 2 sentinels per group, and one group of 2 unvaccinated controls. All animals were challenged at day 21 post-

vaccination. The basic design of this study was similar to that of other studies and observations and samples were collected in a comparable manner. Each of the vaccines studied had been produced on a pilot scale and each contained only 8 µg E2 antigen per ml. Consequently, the dose administered was 4ml and this contained twice the normal amount of adjuvant. The data are therefore supportive of stability but should not be considered as definitive evidence upon which a shelf-life decision should be based.

An increase in body temperature was seen in the majority of vaccinated pigs (14/24 > 40.5°C) following challenge and this was associated with depression and diarrhoea. The sentinels in groups A and C showed no evidence of infection but one sentinel in group B exhibited a marked increase in body temperature. Since this animal did not exhibit detectable viraemia or viral antigen in the tissues the Applicant has concluded that this observation was due to an intercurrent infection. This may be true but the timing of the appearance of the increase in body temperature is consistent with CSF infection from the vaccinates and there are many vaccinated animals which exhibited similar increase in body temperatures without showing detectable virus. It is therefore unsafe to conclude that transmission to this animal did not occur even though there was no evidence of leucopaenia or thrombocytopaenia in the vaccinates or sentinels.

Virus was not isolated from the leucocytes of any of the vaccinates or sentinels at any time after challenge. However, it should be noted that the sensitivity of the assay was, at times, rather poor and it appears that only titres > 2.55 log₁₀ could be detected on some occasions. Since the control animals exhibited titres > 4 only a few days prior to death these data do not indicate complete freedom from infection amongst the vaccinates. It is, however, true that viral antigen could not be detected in tissue samples from any vaccinate or sentinel at slaughter whereas all tissues from the controls were positive. This does suggest that even if infection did occur the animals were sufficiently immune to overcome it.

The immune response elicited by these stored vaccines was generally comparable to that exhibited by the same vaccines in other studies. All the vaccinated animals showed detectable responses following vaccination but three of the vaccinates in group A showed only weak and rather delayed anamnestic responses to challenge. This is probably a reflection of variation in the intranasal challenge since the two worst serological responders also happened to have shown the least evidence of increase in body temperature. Certainly it does not indicate any intrinsic defect in the vaccine.

Whilst it is reasonable to conclude from these data that stored vaccine is equally efficacious compared to freshly prepared product, the design of the study does not facilitate the demonstration of minor changes in potency. In order to achieve this it is necessary to examine the responses obtained in the linear part of the dose regression. Consequently, in the subsequent study performed using the same vaccine batches after storage for 21 months four different doses of one batch of vaccine together with the normal dose of the other two batches were used. Sensibly enough, the Applicant selected the batch used in group B above (batch 95/E2-4) for the dose regression element of the study. In other respects the study was performed in a similar manner to that described above.

In the second study, 50 SPF pigs aged 6-7 weeks were divided into six groups of 6 vaccinates (doses: A;1, B;4, C;16, and D,E,F: 32 µg), plus 2 sentinels per group, and one group of 2 unvaccinated controls. Except for the sentinels, pigs were challenged 3 weeks after vaccination. Batches used were different among groups D (95/E2-4), E (95/E2-3 monolayer), F (95/E2-5 monolayer). As would have been expected those animals, which received the lower doses of vaccine, were clearly more seriously affected by challenge than those receiving the normal dose and some which received only 1 or 4 µg of vaccine failed to survive the challenge.

All the animals, which received less than the normal dose of vaccine, showed increase in body temperature, which with a single exception exceeded 41.0°C. Furthermore, sentinels in groups A and B also showed marked increase in body temperature. Significant leucopaenia and thrombocytopaenia was also recorded amongst both the vaccinates and sentinels of groups A and B but was recorded only infrequently for any of the other vaccine groups. Both viraemia and viral antigen were detected amongst

the vaccinates and sentinels in groups A and B. One sentinel animal in group E showed a marked leucopaenia immediately prior to sacrifice and also showed a very clear increase in body temperature indicating that transmission may have occurred. Examination of the virus isolation results confirms that this animal was indeed viraemic. Furthermore both the sentinel animals in group E displayed viral antigen in the tissues at slaughter.

In contrast, none of the animals which received the full dose of either of the other vaccines or one half the normal dose of vaccine batch 95/E2-4 showed any evidence of viraemia or viral antigen and all the sentinels associated with these groups were also negative.

Examination of the serological results from this study confirms that all the animals which received 16 µg or more of antigen possessed detectable titres at the time of challenge and all were able to mount rapid and important anamnestic responses. In contrast, those animals which received only 1 or 4 µg of antigen had either no or very low titres at challenge although the survivors had mounted anamnestic responses. Again these observations are entirely consistent with the results obtained in earlier studies with the same vaccine.

It therefore appears that in this study vaccine batch 95/E2-3 failed to provide complete protection against transmission. In contrast, the other batches of vaccine both gave protection equally as well as freshly prepared vaccines. In particular, the results obtained with batch 95/E2-4 indicate very clearly that the protection afforded was better than that seen when the vaccine was tested after 12 months storage and at least equivalent to that recorded in earlier trials. These results are in fact, not inconsistent because there is evidence from several of the studies that occasional animals will transmit infection despite appearing to have developed good immunity.

The data support the fact that vaccination reduces the risk of horizontal spreading. Therefore, in the SPC, it is stated that this vaccine is intended for active immunisation of pigs, over the age of 2 weeks, against Classical Swine Fever (CSF), to prevent mortality, reduce clinical signs of the disease and the excretion of field virus.

D. FIELD TRIALS

A true field trial for efficacy of CSF vaccines cannot be conducted within the EU because any outbreak of disease would necessitate the slaughter of all the animals. The presented studies are focused on serological data.

In the first study discussed above in section C5, conventionally reared animals were used, e.g. held in normal farm conditions until the time of slaughter. The data from this study therefore indicate that the immunological performance of vaccinated animals in the field is comparable to that of those maintained under laboratory conditions.

The Applicant has also conducted a limited field study on one farm in which a large number of animals of various categories were used and in which both single (D0) and double vaccination (D0 and 42) was employed. A substantial amount of evidence for vaccine safety has been derived from this study and it is particularly important that the study included very young piglets both with and without maternal antibody. Efficacy within this study has been assessed principally by serology. The study was performed in Germany in an integrated SPF pig farm that had no history of infection with Classical Swine Fever. All the vaccinated animals responded to vaccination.

The fattening pigs, vaccinated with a single dose at 10 weeks of age responded within 4 weeks and maintained their titres with only a small decrease over the following 3 months.

Sows and gilts vaccinated as adults up to the age of 40 months also responded. Those administered two doses of vaccine showed a clear anamnestic response to the second dose but even those given only one dose developed high titres which were maintained for more than 6 months.

In piglets vaccinated once only at 2 weeks of age in presence of maternal antibody titres were maintained at a high level for up to 24 weeks. When these very young piglets were given two vaccine doses (at 2 and 8 weeks of age) there was a very clear and rapid anamnestic response following the second dose and the titres achieved were substantially greater than those in the single vaccination group. However, by six months of age these titres had declined and were only marginally greater than those of animals which had received a single dose. Again the responses were unaffected by the presence of maternal antibody. It is particularly important to note that maternal antibody in the placebo treated group actually declined quite rapidly and did not seem to enhance the response to vaccination at any time. On the basis of these data it would seem that a single dose regime would, in fact, be effective even in these very young animals. However, given the significantly greater titres achieved following revaccination the recommendation for two dose vaccination is a wise precaution and will ensure that any individual animals which may not be totally immunocompetent at the time of the first vaccination will be protected. A small number of these animals were, in fact, challenged within the first study mentioned in this section D. It is worth noting that although the mean titres of the single vaccination group indicate that protection would be expected there was one animal which, when challenged, was still able to transmit virus to other susceptible animals and was itself clearly infected. This observation tends to reinforce the value of a two dose vaccination regime, as recommended, for these young piglets.

The titres of neutralising antibodies differ between the laboratory used in this study and the laboratory used in laboratory studies. Between test (intralaboratory) variation showed differences of approximately 0.5 log due to biological properties. The additional variation might be caused by differences in the test performance and conditions (e.g. predilution of sera, cell culture or indicator virus used). Taken into account the results of a comparison between the two laboratories, it can be concluded that data from these studies are in line with the results obtained following vaccination under laboratory conditions.

RISK-BENEFIT ASSESSMENT AND CONCLUSION

The data submitted in the dossier and in response to questions confirm the acceptability of the proposed formulation and presentations, the choice of the antigen, the method of manufacture of the product and the validity of the test methods applied to the product. The stability tests provided for the finished product show that the product is stable for 14 months. The retained in-use shelf life is 8 hours.

The studies on the safety of the administration of one dose were performed on 6-7 weeks old piglets. Those studies show that the administration of the vaccine can induce systemic reactions such as vomiting, diarrhoea, anorexia, depression and transient increase in body temperature. Local reactions (transient swelling) can occur. Microscopic examinations have shown that in many cases, granulomatous reactions and abscesses will develop and persist at the injection site. The safety of the administration of an overdose has been performed in all categories of pigs and is considered acceptable. No other side effects than those recorded using one dose have been recorded, except that the swelling at the injection site can be observed for up to 2 - 3 weeks. Studies on safety after repeated administration of one dose showed that the repeated administration in piglets and in finishing pigs can induce an increase in the rectal temperature following the second injection. No other signs of systemic reactions were observed. As in other studies, macroscopic examinations showed that in most animals granulomatous reactions and abscesses are detectable and are likely to persist until slaughter.

Taking into account the importance of the disease, the benefits of the vaccination should outweigh the risk and the local reactions and lesions observed during the safety tests are therefore considered acceptable.

An extensive description of the possible local reactions has been introduced in the SPC under 5.3 Undesirable effects.

The vaccine has been administered to gilts and sows at all reproductive stages. No negative effects on reproductive performance have been recorded. The SPC therefore indicates that the product can be used during pregnancy.

Bayovac CSF E2 does not contain live organisms. There is no risk for the environment associated with the use of the vaccine.

Efficacy trials with Bayovac CSF E2 were performed in piglets. The recommended dose of E2 antigen (32 µg) has protected piglets against mortality induced by a CSF challenge. Animals challenged two weeks after one single injection were protected against mortality and showed a reduction of clinical signs and viral excretion. The duration of immunity of 6 months has been supported by specifically designed studies.

The vaccine has been shown to prevent transplacental transmission of low virulence strains after two vaccinations of the sow. However, it may not prevent transplacental transmission of highly virulent strains from the sows to the foetuses. This has been highlighted in the SPC under 5.5 Pregnancy and lactation. One study showed that E2 antibodies can be transferred by colostrum. These maternally antibodies, however, are not protective against mortality after challenge with a virulent CSF strain five weeks post-partum. The single vaccination with E2 vaccine induced clinical protection against CSFV. The vaccination could prevent viraemia and virus spread to the organs, but not completely prevents transmission of virus to highly susceptible sentinel piglets. A second study showed that vaccination, at the age of 2 weeks followed by the second vaccination at 8 weeks of piglets born to previously vaccinated sows partially confers protection against a challenge performed at the age of 6 months. The absence of influence of maternal antibodies on vaccination has been proven.

Prevention of horizontal virus spreading has been carefully studied using sentinel animals. It can be concluded, that the horizontal spreading is reduced.

A field trial using serological responses of vaccinated animals has been performed, keeping in mind that field trials with field virus challenge were not allowed by veterinary sanitary authorities.

In addition the Applicant has demonstrated that already vaccinated pigs are still able to produce anti E^{RNS} antibodies especially when exposed to strains of low virulence.

In conclusion, the product showed efficacy in demonstrating active immunisation of pigs, over the age of 2 weeks, against Classical Swine Fever (CSF), to prevent mortality, reduce clinical signs of the disease and the excretion of field virus.

Based on the original and complementary data presented the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC.