SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER

Improvac is a solution for injection with a minimum of 300µg conjugate active substance per 2 ml dose, intended as an alternative to physical castration for the reduction of boar taint, caused by the key boar taint compound androstenone, in entire male pigs following the onset of puberty. Another key contributor to boar taint, skatole, may also be reduced as an indirect effect. The active substance of Improvac is a Gonadotropin releasing factor (GnRF) analogue-protein conjugate, which is a synthetic peptide analogue of GnRF conjugated to Diphtheria Toxoid.

The benefit of Improvac is the induction of antibodies against GnRF to produce a temporary immunological suppression of testicular function. This allows its use as an alternative to physical castration for the reduction of boar taint caused by the key boar taint compounds, androstenone and skatole, in entire male pigs following the onset of puberty. The most common side effects are injection site swellings of up to 4x8 cm, when administered to pigs at the youngest recommended age (8 weeks) and from 2 to 5 cm in diameter, when administered in older pigs (14-23 weeks). Accidental self- injection may produce similar effects in people to those seen in pigs. The risk of these effects is greater after a second or subsequent accidental injection than after a first injection. Therefore the product must only be used with a safety vaccinator which has a dual safety system providing both a needle guard and a mechanism to prevent accidental operation of the trigger.

2. QUALITY ASSESSMENT

Composition
Improvac is presented as a solution for injection in vials containing 2 ml per dose. The active substance is a synthetic peptide analogue of GnRF conjugated to a carrier protein.

The qualitative composition is given in the table below:

Names of ingredients	Function
GnRF analogue-Diphtheria Toxoid conjugate	Active ingredient/Antigen
DEAE-dextran	Adjuvant
Thiomersal	Preservative
Water for Injections	Diluent
Urea	Solubilising agent

Container
The container consists of colourless high density polyethylene vials of 20 ml (10 doses), 100 ml (50 doses) or 250 ml (125 doses) sealed with a chlorobutyl rubber stopper and secured with an aluminium cap. The vials are in compliance with Ph.Eur. monographs 3.2.2. The stoppers are in compliance with Ph.Eur. monograph 3.2.9. The vials are gamma-irradiated and the stoppers are steam sterilised.

Specifications, drawings and representative certificates of analysis were provided. The additives in the HDPE-bottles were specified and were in compliance with Ph.Eur. 3.1.5. The stoppers are siliconised and are purchased already washed and siliconised. A statement from the supplier was given stating that the silicone oil meets the requirements of the Ph.Eur. monograph for dimeticone.

Development Pharmaceutics

Choice of antigen:
The antigenic component is a synthetic analogue of the natural 10-amino acid peptide, gonadotropin releasing factor (GnRF), covalently conjugated to Diphtheria Toxoid. The conjugated component stimulates an active immune response against endogenous GnRF and interrupts the production and/or accumulation of boar taint compounds. The GnRF peptide analogue contains the critical epitope(s) necessary to stimulate an effective anti-GnRF antibody response. The synthetic peptide analogue has been designed to contain the critical region necessary for the targeted immune response, while making it suitable for conjugation and essentially devoid of hormonal activity. The peptide analogue is too small to be an effective immunogen, and needs to be coupled to a larger immunogenic molecule in order to create an antibody response. Diphtheria Toxoid is a stable and safe, highly immunogenic substance that delivers this effect. Diphtheria Toxoid is used for vaccination of humans and as carrier protein in vaccines for human use. The conjugation chemistry was selected to provide a highly stable linkage between the peptide and the carrier protein, thus contributing to the stability of the product.

Choice of solubilising agent:
GnRF analogue-Diphtheria Toxoid conjugate has limited solubility. A solubilising agent was chosen to ensure solubility during the conjugation reaction, ultra filtration, sterilisation before blending, and to ensure homogeneity during conjugate testing and final product blending activities. This solubilising agent is a naturally-occurring, physiologically-compatible biological compound known to be acceptable as an excipient in vaccine formulations. It was proven as an effective solubilising agent. Its suitability has been subsequently demonstrated in the efficacy and safety studies.

Choice of conjugate quantity:
The dose of conjugate was selected on the basis of several dose regression studies in pigs.
A serological response of 474 U/ml was defined as the minimum acceptable serologic response.

Choice of batch potency assay:
In addition to an HPLC assay to measure the conjugate content in the finished product, an in-vivo assay using the target species was developed.

Choice of adjuvant:
DEAE-Dextran was selected as the preferred adjuvant as it was demonstrated during the early developmental work to be highly effective and well tolerated when injected into animals. The dosage of DEAE-Dextran that is used in Improvac was selected as this specific dosage was found to produce a highly consistent response and minimal local reactivity at the site of administration. The compound is easily managed within the manufacturing environment and can be accurately measured in final product using a conventional, validated method (refractive index).

Choice of preservative:
Ph.Eur. grade Thiomersal was chosen as preservative in the multi-dose containers. The preservative meets the preservative efficacy requirements for both bacteria and fungi as defined in Ph.Eur.

Choice of containers and stoppers:
The containers used for Improvac are plastic multi-dose vials made of High Density Polyethylene (HDPE) material, closed with chlorobutyl flat stoppers and sealed with aluminium caps.
The choice of container is based on well-established use of this type of container for veterinary medicinal products.

Overage:
Improvac will be available in 10, 50 and 125 doses presentations. The minimum fill volume for all presentations is the nominal volume plus 5%. This volume ensures that the stated number of doses can be recovered from the container. The minimum Diphtheria Toxoid-GnRF analogue conjugate content of 0.3 mg/dose has been set to ensure that commercial batches will still meet the final product specification for host animal potency at the end of shelf-life.

Preservative efficacy:
Preservative efficacy was tested according to Ph.Eur. by direct inoculation of micro-organisms into vials. Criteria A were met for *Pseudomonas aeruginosa, Candida albicans and Aspergillus niger.* Criteria B were met for *Staphylococcus aureus.*

Composition of the batches used in the clinical trials
The details of the batches used in the pivotal clinical trails were provided. The same composition as intended for marketing was used with the exception of conjugate content which was justified.
Only one combination of diphtheria toxoid (DT) and GnRF peptide analogue starting material suppliers will be used for the production of commercial Improvac batches for the EU. Improvac batches used in the submitted safety and efficacy trials used different starting material supplier combinations and were all found equivalent to the batches from the above commercial manufacturers.

METHOD OF MANUFACTURE
Detailed description of the manufacturing method was provided together with the relevant flowcharts.

Diphtheria Toxoid-GnRF analogue conjugate:
Detailed technical information as requested by the CVMP was provided by the Applicant regarding the different steps of the process.

In process controls:
The CVMP considered that relevant in-process controls had been indicated appropriately.
Information of holding times was also provided and justified.

Overall the CVMP considered that the manufacturing methods were described in sufficient details and were satisfactory.

II.B.3 Validation studies

Consistency of all steps of manufacturing was demonstrated by specification testing of three consecutive batches. The proposed storage period of the GnRF analogue-DT conjugate was 12 months. Data were generated to support this.

STARTING MATERIALS

Listed in a Pharmacopoeia
The following comply with the European Pharmacopoeia:

Diphtheria Toxoid (DT)
Disodium Edetate (EDTA)
Disodium Phosphate, Anhydrous
Hydrochloric Acid, Concentrated
Sodium Dihydrogen Phosphate
Sodium Hydroxyde
Thiomersal
Urea
Water for Injections (WFI)

Dimethyl Sulfoxide (DMSO) also complies with Ph. Eur except for its refractive index.

The starting materials comply with the current editions of the Ph.Eur. A representative certificate of analysis for the above starting materials is provided.

As stated earlier only one combination of diphtheria toxoid (DT) and GnRF peptide analogue starting materials will be used for the production of commercial Improvac batches for the EU. Information for all the alternative starting material manufacturers responsible for the batches that were used in the pivotal efficacy and safety trials was submitted. The information was assessed and equivalence of those batches to the commercial batches was established. In this section only the information related to the manufacturers involved in the commercial combination was presented.

Not listed in a Pharmacopoeia
Starting materials of biological origin

Diphtheria Toxoid:
Diphtheria Toxoid complies with Ph.Eur. monograph 0443 "Bulk Purified Toxoid". The Diphtheria Toxoid is produced from the master seed. The history of the strain was described in detail.

Manufacturing process
Updated flow-charts and detailed description of the production process of the toxoid were provided.

Process validation (PV)
Results for process validation conducted on three consecutive production batches were presented. In summary, each process validation batch met or exceeded all the in-process and final DT product testing specifications. Thus, it was concluded that the DT manufacturing process is capable of consistently producing a product meeting predetermined specifications and quality characteristics. Therefore, the manufacturing process was considered validated in accordance with current regulatory requirements.

Detoxification (Toxoiding) Process Validation
The detoxification (toxoiding) process validation (PV) kinetics were carried out on three consecutive production batches..
The detoxification step was considered critical by the CVMP for the quality of the diphtheria toxoid and therefore full scale validation data on the detoxification step were provided (e.g. kinetic study of the detoxification). The kinetic study demonstrated that the complete detoxification is achieved in less than 67% of the time used during production and the validation of the detoxification process is therefore considered acceptable.

Overall, the manufacturing process was considered validated.

Starting materials used in the manufacturing of DT
All starting materials of non-biological origin were listed, with reference to Ph.Eur/USP or in-house reference, respectively.

In relation to starting materials of biological origin the following were used:
Master seed:
A detailed description of the seed history and batch records indicating the seed used to produce the master and working was provided. The master seed lineage was reviewed and found acceptable.

Working seed:
Master seed is used to produce *C. diphtheriae* Working Seeds *(*WSB). Working Seeds are stored at -70°C or below until used for further production. The following tests are performed on the working seed lots: purity, identity, biochemical tests and colony morphology.
Updated information provided by the Applicant on the seeds, including controls, was considered acceptable.

A note stating that the ingredients of ruminant origin are being sourced from certified vendors sited in TSE & BSE free/low risk countries was included in the Specifications of raw materials of ruminant origin. Further the Application provided a risk analysis for viral contamination. Based on this analysis the CVMP concluded that the selection and control of the starting materials was acceptable.
Preparation and storage for media and solutions were stated. All media and solutions are sterile filtered and tested for sterility (where applicable).

Control tests during production
The following in-process controls are performed: culture purity, toxoid content, total protein nitrogen, antigen purity, free formaldehyde and bioburden.

Control tests on the bulk product
The following tests are performed on the Diphtheria Toxoid bulk product and the proposed acceptance criteria were considered adequate. Tests: Appearance, Sterility, pH, Toxoid Content by Flocculation (Lf/mL), Total nitrogen, Protein nitrogen, Antigenic purity, Free formaldehyde, Absence of Toxoid, Irreversibility of Toxoid, Identity.
Sufficient information was provided on the test and the method descriptions.

Batch to batch consistency:
Batch results were provided for three batches of Diphtheria Toxoid. All batch results complied with specification. Consistency between batches was shown. On the basis of the stability data presented a 12 month shelf life was granted.

DEAE-Dextran:
One of the materials in DEAE-Dextran is dextran, which is produced using skimmed milk powder. The milk is sourced from healthy animals under the same conditions as milk collected for human consumption. No other material of ruminant origin is used in the preparation. Information on TSE risk is provided in Part 2.D. Specifications and a representative certificate of analysis were provided. The specification tests for DEAE-Dextran includes a test for appearance, identification, degree of substitution (nitrogen content), specific optical rotation, loss on drying and pH. The DEAE-Dextran specifications have been modified to include tests for microbial contamination and bacterial endotoxin content. The specification for DEAE-Dextran was considered acceptable to ensure the chemical quality.

Starting materials of non-biological origin
Some starting materials of non-biological origin are used which do not have a compendial monograph. Specifications and representative certificates of analysis were provided for all starting materials.

GnRF peptide analogue:
The substance is not described in the Ph.Eur. The list of all the tests to be performed on each batch of peptide before its release for use in manufacturing and the acceptance criteria were provided. Updated specifications were provided and were found acceptable. The Applicant acknowledged that the peptide is a critical intermediate in the manufacturing of the active ingredient (Diphtheria Toxoid GnRF analogue conjugate). Currently the Applicant performs retests on all batches of peptide. This was considered acceptable.

The GnRF peptide analogue is manufactured by a classical organic synthesis process. A synthesis flow sheet was provided. Adequate in-process control was applied. Reproducibility of the process was demonstrated.

The purification scheme was presented in detail. For the purification step, details of the columns used were provided. The purity of the acceptable fractions was indicated together with the methods used to determine this purity. Details of the freeze-drying processes, particularly the final freeze-dry step were also provided.

Raw materials, solvents and reagents

Specifications on raw materials, solvents and reagents were submitted. All materials used in the production process are sourced from approved suppliers. The materials were provided with Certificates of Analysis (or Technical Specification sheets), and were subject to documented procurement, acceptance, testing and storage procedures.

Characterisation

The chemical structure was confirmed by amino acid analysis, MS, N-terminal sequencing and optical rotation. HPLC testing has also been performed using the established reference standard.
Details of the origin and characterisation of the reference standard were presented.

Impurities

Related Substances

No specific intermediate products are isolated or characterised during synthesis. The structures of the impurities have been elucidated following determination by MS and subsequent synthesis of the proposed structures.

Residual solvents

A number of solvents were used throughout the synthesis process. Overall, the levels of residual solvents in final GnRF peptide analogue were shown to be reduced to satisfactory levels. The test method used to control residual solvents was described and shown valid in accordance with VICH guidelines.

Batch analysis data

Data were provided for 6 batches showing compliance with the proposed specification and consistency across batches.

Specifications

The specifications were updated to include limits on individual and total related substances, residual solvents, water content, TFA content, pH, mass balance and bacterial endotoxins.
The CVMP considered that a retest period of 6 months is acceptable.

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

A declaration of compliance with the Note for Guidance (NfG) on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01 rev 2) was provided.

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

CONTROL TESTS DURING PRODUCTION

The control tests performed on the Diphtheria Toxoid-GnRF Analogue Conjugate: i) Epitope Density, ii) DT-GnRF Analogue Conjugate Content by RP-HPLC, iii) pH, iv) Sterility.
Detailed description and method validation data for these tests were provided. The DT-GnRF analogue conjugate content RP-HPLC test is also used for the finished product.

Epitope density test:
This test involves a reverse phase HPLC column with gradient acetonitrile elution and fluorescent detection monitor. Samples are fully hydrolysed and derivatised before determination of amino acid content by HPLC. An internal standard is used as well as a calibration standard. Good repeatability, intermediate precision and reproducibility were shown.
The epitope density specification limits were considered satisfactory.

DT-GnRF analogue conjugate content RP-HPLC test:
The RP-HPLC test was validated with regard to linearity, range, precision (repeatability and intermediate precision). Linearity and precision were demonstrated for both the active substance and the finished product.
Additional details on the Reverse Phase HPLC method were provided together with the updated validation of the method in order to provide guarantees for the robustness and consistency of the method used for conjugate quantification.
Specificity
The specificity of the method was investigated by individually testing the different analytes present in the final product using the RP-HPLC method to determine their elution position. Acceptable data were provided to confirm the specificity of the method.
Accuracy
The accuracy of the method was estimated by comparing a theoretical input with the returned result at both the conjugate and final product stages.
Potential Impact of Epitope Density:
It was clarified that it was not expected that the ED of the conjugate on test will greatly influence the results of the RP-HPLC assay, and therefore the epitope density was not considered as part of the RP-HPLC validation.
Reference standard
The Applicant provided adequate information on the reference standard used in the RP-HPLC method.
Specifications:
The conjugate content should be a minimum of 3000 µg/ml.

Conclusions:
The CVMP considered that adequate information on the RP-HPLC method had been provided.
The accuracy and specificity of the method was successfully validated. The epitope density was not considered as part of the validation of the RP-HPLC method. In conclusion the RP-HPLC method was considered sufficiently validated and the specification limits are acceptable based on the presented batch results.

Sterility test:
The sterility test was validated according to Ph.Eur monograph 2.6.1 for the membrane filtration method on three batches.

pH test
Method validations were performed for all specification tests except for the pH test, which was acceptable.

Batch to batch consistency
Batch protocols for three consecutive batches of Diphtheria Toxoid-GnRF Analogue Conjugate were provided. The results were consistent and all results comply with the specification.

CONTROL TESTS ON THE FINISHED PRODUCT

The tests performed on the finished product specification are listed in the table below. The specifications for each test were provided and were considered satisfactory:

Tests
Description
Identification
Sterility
Safety test in pigs
Potency by RP-HPLC
Potency test in pigs
Refractive Index
pH
Thiomersal
Individual volume

Detailed description and method validation data for the sterility test, potency by RP-HPLC, pig potency, refractive index (DEAE-Dextran content) and thiomersal test were provided. Method descriptions were provided for the safety test in pigs and the test for volume. The test for appearance of the final product was included and the acceptance criteria further defined as follows: "Plastic vial containing a clear, colourless to yellowish viscous solution". The necessary checks are also in place to ensure that no mix-ups in the colour of the aluminium caps in the finished product may occur.

Safety test in pigs:
2 doses of Improvac are injected subcutaneously into normal, healthy non castrated male pigs of 8 to 16 weeks old. The pigs are observed for 14 days.

Potency by RP-HPLC:
The CVMP concluded that satisfactory data on the RP-HPLC method for the control of the finished product were provided. The accuracy and specificity of the method were successfully validated. In conclusion the RP-HPLC method was considered sufficiently validated and the specification limits are acceptable based on the presented batch results

In vivo potency test in pigs/potency by ELISA:
Overall, sufficiently detailed information on the pig vaccination*, ELISA serology, and assay standards were provided.

The CVMP considered that the ELISA test has been validated with regard to accuracy, repeatability, intermediate precision, specificity, linearity and range. The validation is considered to be in accordance with the VICH guidelines on validations of analytical procedures. The variability of the potency test implied that the potency test can not be use as a stand alone test to ensure consistent potency. The pig potency assay is therefore complemented by in vitro assays; epitope density, conjugate content and adjuvant content. The combination of the pig potency test, epitope density test and conjugate test will ensure the potency and that no sub potent batches are released. In the context of the rationale above, the pig potency test can be regarded as a confirmation of immunogenicity but provides little reassurance of equivalence with the batches used in efficacy studies. It is therefore

necessary to put more reliance on the epitope density and conjugate content assays as reliable parameters for batch release.

*As Improvac is an immunological product with a similar action mode to that of a vaccine (stimulates the immune system to produce antibodies), the terms "vaccination" and 'immunisation' have been used in this report to describe the injection of animals to illicit an immune response to the product. It was considered a more "user friendly" term within the context of the mode of action.

Thiomersal:
The mercury in the sample is oxidized to Hg^{++} following reduction to metallic mercury. The mercury vapour is quantified by Atomic Absorption Spectrometry according to the direct calibration method described in Ph.Eur. 2.2.23. The test was successfully validated with regard to accuracy, linearity, range and precision (repeatability and intermediate precision).

Individual volume/extractable volume:
The method is based on the extractable volume test described in Ph.Eur. Monograph 2.9.17.

Sterility test:
The sterility test was validated according to Ph.Eur monograph 2.6.1 for the membrane filtration method on three batches.

Refractive index (DEAE-Dextran content):
The test was performed according to Ph.Eur. 2.2.6. The test was validated with regard to linearity, accuracy, range, precision (repeatability and intermediate precision).

Batch to batch consistency
Batch release protocols for three consecutive batches of Improvac filled in 10, 50 and 125 dose vials were provided. The results were consistent and all results comply with the specification.
Process Validation
In order to validate the batch to batch consistency of conjugate manufacturing process and to generate stability data, validation batches of conjugate were produced
All these Improvac batches complied with the release specifications and demonstrated the batch to batch consistency in the manufacturing process.
Conclusions:
The Applicant proposed a maximum batch size for the conjugate and the finished product which were considered acceptable.

STABILITY

Stability of the active substance
On the basis of the submitted data the CVMP considered that a shelf life of 12 months at 2-8°C was acceptable.
Stability data from the following studies were presented: real time stability study at 2-8°C for 24 months, and accelerated stability study at 20-25°C for three days followed by 2-8°C for 24 months, Real-time stability data were also provided for batches with active substance stored for 6 and 12 months. The CVMP concluded that testing performed every 6 month during the 24 months stability studies is adequate to evaluate the stability of the active substance.
Moreover, only one starting material combination was used in the stability studies. The Applicant commited to perform stability studies with batches of DT and peptide used in the commercial product.

Stability of the finished product
On the basis of the submitted data the CVMP considered that a shelf life of 24 months for the finished product is acceptable. The Applicant justified satisfactorily that no test for impurities or degradation products were included in the shelf-life specification. They explained satisfactorily that no test is performed for process related impurities.

The testing frequency was considered acceptable. Stability data for up to 27 months were provided for six primary stability batches and results were within the the limit of the specification. Based on the 27 months stability data a shelf life of 24 months was approved.

The sources of diphtheria toxoid and peptide used in the batches of Improvac submitted to storage were specified. The variability of the antigenic content such as controlled by the HPLC method was addressed satisfactory.

In-use shelf-life
The efficacy of the preservative has been shown at release and after in-use storage (10 hours at room temperature and at 2-8°C). An in-use shelf life of one working day (8-10 hours) was acceptable based on the in-use shelf-life presented.

OVERALL CONCLUSION ON PART II ASSESSMENT

Improvac is presented as a solution for injection in vials containing 2 ml per dose. The active substance is a synthetic peptide analogue of GnRF conjugated to a carrier protein (Diphtheria Toxoid). Other ingredients in the final product are: DEAE-dextran, Thiomersal and water for injection. As Improvac batches used in the submitted safety and efficacy trials were all manufactured using alternative starting material manufacturers from the ones to be used for commercial purposes the Applicant had to demonstrate equivalence between the different combinations of conjugates. The statistical comparison of pig potency results show that there was no significant difference between batches manufactured with conjugate for commercial batches and conjugate used in the clinical studies and batches used in the stability programme. This was supported by results of the non-inferiority study using the different conjugates.

DEAE-Dextran and DT are the only two starting materials which are derived from materials of ruminant origin. Based on the risk assessment the overall the TSE risk of Improvac is considered minimal.

An epitope density test is performed on the DT-GnRF analogue conjugate. Based on batch results of conjugate batches the Applicant proposed specification limits for the epitope density test that are acceptable according to the safety and efficacy trials.
A pig potency test is performed on the finished product. The specification limit is ≥ 474 U/ml. To ensure consistent potency, the pig potency assay is complemented by the in vitro assays; including epitope density, conjugate content and adjuvant content. The combination of the pig potency test, epitope density test and RP-HPLC GnRF analogue-DT conjugate test will ensure the potency and that no sub potent batches are released. The Applicant has shown by titration studies that the RP-HPLC GnRF analogue-DT conjugate test ensures that no sub-potent batches will be released. The release specification limits for the RP-HPLC GnRF analogue-DT conjugate have been set to ensure a minimum of 300 µg/dose at the end of shelf-life.

The following shelf-lives have been approved:
GnRF analogue-DT conjugate: 12 months at 2-8°C.
Finished product: 24 months at 2-8°C for the 10, 50 and 125 dose vials.
In-use shelf-life: 10 hours.

3. SAFETY ASSESSMENT

INTRODUCTION

Improvac is an immunological product comprising a conjugate of a synthetic peptide analogue with diphtheria toxoid, combined with an adjuvant (DEAE-Dextran). The Applicant has presented studies carried out under laboratory and field conditions to demonstrate the safety in the target animal i.e. non-castrated male pigs, together with implications for operator safety, consumer safety and the environment. It is considered that Improvac provides a safe alternative to surgical castration through the process of immunisation against endogenous GnRF when used as recommended in the SPC.

The main risk for human safety is recognised to be from accidental self-injection with the potential to affect fertility in men and women, especially during pregnancy. Although special equipment has been developed, self injections may occur and therefore great care should be taken when administering this product. The first injection appears very rarely to give any change in anti-GnRF titre or serum testosterone (one case report out of three after use of over 7 million Improvac doses, the incidence of self injections being 0.00004%). This is supported by results from humans with prostate cancer treated with a similar product. Furthermore, it appears that if a chance self-injection occurs the effect will remain for a limited period only. During this period it is possible to provide the self injected human patient with a supportive therapy. Residue intake is not of relevance since the product is destroyed in the gut and is not immunogenic by the oral route (pig and rat experiments).

GENERAL REQUIREMENTS

The studies regarding safety in target animals were all conducted in accordance with: Directive 2001/82/EC amended by Directive 2004/28/EC European Pharmacopoeia 5.2 Section 5.2.6, EOCD Principles on Good laboratory Practice (GLP), CVMP-VICH GL 9 on Good Clinical Practice (GCP), Guidelines for the production and control of pig live and inactivated vaccines and in consideration of CVMP/543/03 (Guideline on User Safety).

A. SAFETY ASSESSMENT

LABORATORY TESTS

Safety of the administration of one dose, an overdose and repeated administration of one dose

A GLP study included animals which were administered single, overdose and repeat doses of Improvac. The batch was targeted to contain 400 µg/ml GnRF conjugate (twice the target concentration) but the certificate of analysis indicated that it contained only 348 µg/ml. This was accepted as a high antigen concentration batch suited for this use because it still exceeded the maximum antigen content specified for the product.

Study design

Cross-bred male pigs, 8 weeks old at Day 0 were divided into 4 groups. At Day 0 one group was administered a single dose (2 ml) subcutaneously into the neck; the second group was administered a double dose (4 ml); the third group was administered a single repeated dose at Days 0, 14, and 28. The fourth group acted as control being administered a double dose of sterile saline at Day 0 followed by single doses at Days 14 and 28.

Follow up

Rectal temperatures were measured daily at Days -1, 0, and 1-14. Injection site reactions were measured at Day 0 +4-6h after injection, and daily from Day 1-14. Any pig with palpable reactions at day 14 was monitored until the second day that no palpable reaction was observed or until termination of the study on Day 42. Clinical examination was made on individuals one hour after each injection and daily at Days 1-14 after each administration. Body weights were measured on Days 0 and 14 for single, double and repeat dose pigs, and these were also weighed on Days 28 and 42 for pigs still

enrolled on these study days. At study termination injection sites of the pigs administered a single dose were examined histopathologically.

Results

Single dose: A transient increase in mean temperature of 0.3-0.6°C was observed during the first 24h period after vaccination Temperatures normalised over the next 2-3 days. Palpable local reactions were recorded in all vaccinates. Twenty per cent of pigs still had these reactions at termination of the study (Day 42). Histological examination revealed inflammation of the subcutaneous tissue in all vaccinates. In thirty per cent of pigs a severe degree of fibrosis was expressed in the deeper adipose tissues indicative of a chronic inflammatory process. No clinical reactions were observed in any animals and weight gains were similar in both vaccinates and controls.

Double dose: A transient increase in mean rectal temperature by up to 0.7°C was recorded during the first 24h period post vaccination. At 24 h post vaccination some saline-treated and some vaccinated pigs had elevated temperatures. All vaccinates had persistent palpable injection site reactions at day 14 post vaccination, when the study was terminated. The largest reaction was 91x47 mm at Day 7 decreasing to 82x33 mm at Day 14. No clinical reactions were observed in any animal and weight gains were equal between vaccinates and controls.

Repeat single dose: A transient increase in temperatures was recorded in both vaccinates and controls on 24h post first vaccination. Twenty four hours after the second vaccination, a transient increase was recorded again. At 24 hours after the third administration similar reactions occurred. All temperatures returned to normal within 2-3 days. Sixty per cent of vaccinates had palpable reactions at day 14 after first vaccination, fifty percent had similar reactions after the second vaccination but no pigs reacted after the third vaccination. No clinical reactions were observed in any pigs and weight gains also were similar in the two groups.

Conclusions

Pigs from the single dose study were euthanased on different days after vaccination depending on the time when local palpation reactions had disappeared. As the Applicant only claimed the product to protect against boar taint for no more than 4-6 weeks after the second injection the safety study was terminated on Day 42. The Applicant interpreted the safety test from Directive 2001/82/EC in a way that pigs should be examined for at least 14 days after injection. It was not agreed by the CVMP that such an experimental design was in the spirit of the Directive. As the second dose of the product was going to be administered 4-6 weeks before slaughter it was felt important to know the expected frequency of injection site reactions. Likewise it was questioned by the CVMP whether Improvac as claimed could be delivered correctly subcutaneously as histological reactions also indicated intramuscular inflammation. The very young pigs (8 weeks) used in this study were also felt not to be representative of the indication for the product, so the Applicant was asked to present further documentation from histological examination on injection site reactions from pigs representing the indicative age. These adverse reactions are reflected in the SPC.

Investigations on injection site reactions in pigs vaccinated at 13-18 weeks of age

Study design

The Applicant provided a study to investigate the histological reactions. Pigs were vaccinated approximately at 13 weeks of age and 30-34 days later. Equal number of pigs were euthanased 2 weeks after the second vaccination and after 4 weeks.

Histological results

It was clearly documented that even in 13-18 weeks old pigs Improvac was delivered into the muscular tissues as well as into the subcutaneous tissue. The product was located some times into the superficial muscle and the overlying subcutis but some times also to the deep muscular block.

Conclusion

The CVMP concluded that it was clear that there was significant involvement of muscular tissue in most cases suggesting that the vaccination method does not reliably deliver Improvac sub-cutaneously.

The Applicant was asked to provide further data on the incidence of injection site reactions. An overview was presented from a total of 572 vaccinated pigs (second vaccination from 28 days to 35 days before slaughter). The total incidence of palpable reactions at slaughter was 27/572 equal to 4.7%. The incidence was therefore be considered as "common" (more than 1 but less than 10 animals in 100 animals) and reflected accordingly in the SPC.

Examination of reproductive performance
Improvac is only intended for use in male pigs intended for slaughter and is contra-indicated for use in breeding animals, hence no studies were performed in female pigs intended for fattening or male and female pigs intended for breeding.

Examination of immunological functions
No specific studies of the impact of vaccination with Improvac on immunological function were conducted. The absence of specific studies was justified by the fact that there was no reason to expect that Improvac or any of its components should induce any adverse effect on immune function. Likewise performance of vaccinates was the same as for non-vaccinates indicating no increased susceptibility to disease. In addition, the carrier protein, Diphtheria toxoid, is widely used in man (especially in children) and would not be expected to induce any immunosuppression.

Interactions
The updated standard wording for immunologicals, Point 4.8 of the SPC was used.

FIELD STUDIES

Safety from the use of Improvac under commercial conditions was provided in two studies, one took place in Spain and another in Germany (both studies were also provided in Part IV of the dossier).

1) Study Title: To confirm the Safety and Efficacy of Improvac Vaccine in Controlling Boar Taint in Male Pigs under Commercial Field Conditions in Spain
The study included pigs on two different farms (Sites). On both Sites the pigs were vaccinated from 14 weeks of age. Animals were randomly assigned to one of three treatment groups as follows:

	Dosage	Regimen	Route of administration
T01	-	Surgical castration	-
T02	2x2 ml	Improvac vaccination	s.c. injection
T03	-	Entire boars	-

Animals were given a subcutaneous immunisation of 2 ml of a minimum potency twice with 4 and 5.5 weeks interval (Site 1 and 2, respectively). The 2nd vaccination was given 4.5 weeks prior to slaughter at both sites. All pigs were weighed at a few days of age and physically examined. Surgically castrated and vaccinated pigs were followed more closely around castration, weaning, and vaccination.

Follow-up:
Rectal temperatures of a sub-set of vaccinates from each site were monitored on D-1, D0, D1-D4 after each vaccination. Injection site reactions in these animals were observed at least one hour before each vaccination, at 4 h after each vaccination, daily from D1-D14, and on the day before slaughter. Injection sites were scored as: 0=no reaction; 1=normal; 2=mild; 3=moderate; 4=severe.
All animals were monitored for clinical signs at least 1 h before each vaccination, 4 h after each vaccination and before slaughter. In addition all pigs in each of the animal sub-sets were monitored for clinical signs daily from D1-D14 after each vaccination. Weights of all pigs in each sub-set were recorded at weaning, D1 after each vaccination, D14 after the second vaccination and one day before slaughter.

Statistical Analysis:
Generalised linear mixed models analysis was used.

Results:
Rectal temperature: None of pigs in groups T01 and T02 had a daily rectal temperature on D1-D4 more than 2°C above their temperature at time of vaccination of group T02. The percentage of pigs that were febrile (one or more days of rectal temperature >40.0°C) following 1st vaccination was: T01= 34.0%; T02= 35.7%. The percentage of febrile pigs after 2nd vaccination was: T01= 13.2%; T02= 12.5%. Most of the pigs with elevated temperatures were febrile only for 1-2 days and results were comparable for the two groups.
Injection site reactions: Injection site reactions were observed in 30% of the pigs after the first vaccination and in 30% pigs after the second vaccination. The presence of injection site reactions was site related. Eighty-five percent of reactions lasted for no more than two days after the first vaccination and 82% of reactions lasted for no more than two days after the second vaccination. Clinically significant reactions lasted for one day in affected pigs. The maximum duration of any reaction was 15 days after the first and 7 days after the second vaccination respectively, with the exception of one animal, in which reaction was still present at slaughter, 29 days after the second vaccination.
Body weight and daily weight gain: Overall there were no statistically significant differences in average daily gain (ADG) between group T01 and T02 after first and second vaccination. Site had a statistically significant effect on ADG from 14 days after the second vaccination to slaughter, but no significant treatment x site interaction affected the ADG.
Clinical abnormalities: None of the recorded clinical abnormalities observed was considered to be a result of vaccination with Improvac, as they were mainly related to the gastrointestinal tract or musculoskeletal disorders. Results were comparable for the two groups.
Mortalities: A total of 9 pigs were euthanased in this study. None of the deaths of three pigs in group T02 was attributable to vaccination with Improvac.

Conclusions:
The Applicant explained any differences as resulting from use of different operators to administer the product, differences in level of hygiene and competence of vaccinators. It was concluded that it is important to adequately educate farm staff before using Improvac. Vaccination had no detrimental impact on body weight or daily weight gain.

2) Study Title: Safety and Efficacy of Improvac Vaccine in Controlling Boar Taint in Male Pigs under Commercial Field Conditions in Germany
This was a GCP compliant study which took place in two sites. Pigs of 19-20 weeks at 1st vaccination were included in site A and similarly, pigs aged 19-21 weeks were inlucded in site B.

In each site animals were assigned to one of three treatment groups:

	Dosage	Regimen	Route of administration
T01	-	Surgical castration	-
T02	2x2 ml	Improvac vaccination	s.c. injection
T03	-	Entire boars	-

Treatment
A subcutaneous injection of 2 ml from a minimum potency batch was given twice with 4 weeks interval in T02 animals. The 2nd vaccination was given 4 or 5 weeks (site A) and 4.5 weeks (site B) prior to slaughter, respectively. All pigs were weighed at a few days of age and physically examined. Surgically castrated and vaccinated pigs were followed more closely around castration, weaning, and vaccination and blood samples were collected, testes and body weight were measured.

Follow-up
Rectal temperatures of a sub-set of vaccinates and surgical castrates at each site were monitored on D-1, D0, D1-D5 after surgical castration. At time of first and second vaccination rectal temperature was measured on D-1, D0 after 1 and 4 hours, D1-D4.
All animals from the sub-set were monitored for clinical signs at the same time as temperatures were measured and were continued from D5-D14 post each vaccination. The day before slaughter, a final clinical examination was performed.
Injection site reactions in these animals were observed at approximately one hour and 4 hours after each vaccination, then daily from D1-D14. If any pig still had an injection site swelling at the end of D14, evaluation was continued on an approximately weekly basis until any swelling had subsided or the animal went to slaughter. Injection sites were scored as: 0=no reaction; 1=normal; 2=mild; 3=moderate; 4=severe.
Weights of all pigs in each sub-set were recorded at study day 0 (prior to surgical castration), at weaning, at first and second vaccination, D14 after the second vaccination and one day before slaughter.

Results:
Rectal temperature: None of pigs in groups T01 and T02 had a daily rectal temperature on D1-D4 more than 2°C above their temperature at time of vaccination of group T02. The percentage of pigs that were febrile following 1st vaccination was: T01= 9.4%; T02= 16.9%. The percentage of febrile pigs after 2nd vaccination was: T01= 5.8%; T02= 0.0%. Most of the pigs with elevated temperatures were febrile only for 1-2 days and results were comparable for the two groups.
Injection site reactions: Injection site reactions were observed in 69% of the pigs after the first vaccination and in 68% pigs after the second vaccination. Sixty-one percent of reactions lasted for no more than two days after the first vaccination and 62% of reactions lasted for no more than two days after the second vaccination. The maximum duration of any reaction was 55 days after 1st vaccination and 34 days after 2nd vaccination. Clinically significant injection site reactions were recorded in 2.3% of pigs from 1-4 hours after the first vaccination. Significant reactions occurred in 28% from 1-14 days after the first vaccination and in 25% after the second vaccination. No significant clinical reactions were observed at slaughter.
Body weight and daily weight gain: At the time of 1st and 2nd vaccination, 14 days after second vaccination and at slaughter, body weights of pigs in groups T01 and T02 were comparable.
Clinical abnormalities: None of the recorded clinical abnormalities observed were considered to be a result of vaccination with Improvac, as they were mainly related to the respiratory tract or musculoskeletal disorders. Results were comparable for the two groups.
Mortalities: Of the euthanased pigs that belonged to group T02, none were were euthanased due to a cause attributable to vaccination with Improvac

CVMP´s conclusion:
Questions were raised regarding the statistically significant different injection site reactions between pigs on the two farms, these were again explained as caused by different dispensers, competence of examiners to assess the injection site reactions. As with the Spanish field study it was concluded that it is important to adequately educate farm staff before use of the product. Vaccination had no detrimental impact on body weight or daily weight gain.

User Safety

INTRODUCTION

The active ingredient (antigen) in Improvac is a synthetic-peptide analogue of gonadotrophin releasing factor (GnRF), conjugated to Diphtheria toxoid (DT). The DT serves as an immunogenic carrier protein. The antigen is combined with DEAE-dextran adjuvant in aqueous solution with thiomersal as preservative.

Two 2 ml doses (300-460 µg/dose) administered at least 4 weeks apart are required to stimulate a strong anti-GnRF antibody response. The first dose acts as a priming dose to the immune system and

has no physiological consequence. After the second dose, antibodies to GnRF are produced which neutralise circulating endogenous GnRF. This neutralisation of circulating GnRF suppresses release of luteinising hormone (LH) and follicular stimulating hormone (FSH) from the pituitary gland which leads to a temporary suppression of testicular function. As a consequence, the accumulation of the boar taint compounds (androstenone and skatole), governed by testicular function, is suppressed and any taint already present at the time of immunisation is eliminated.

The effects of removing GnRF drive to the pituitary in humans are well established through the use of GnRF agonists, antagonists and vaccines. In men, testosterone is lowered to castrate levels, and LH and FSH concentrations remain suppressed. Since testosterone is an aromatisable substrate for oestrogen synthesis by peripheral adipose tissue, circulating oestrogen levels are also lowered by GnRF analogues. Vasomotor symptoms are common as testosterone and oestrogen levels drop. Testis and prostate volume are reduced and sperm count declines. Libido is reduced. Lowered bone mineral density and increased fracture risk is documented in men on prolonged testicular suppression treatment. The effects of testosterone deficiency in men can be overcome with testosterone replacement, but to restore fertility FSH replacement is also needed.

In women, the menstrual cycle is blocked rendering the subject infertile. Estradiol is lowered to the post-menopausal range, and LH and FSH concentrations remain suppressed. Most women experience vasomotor (i.e., menopausal) symptoms and in the longer term develop atrophy of the reproductive organs including thinning and dryness of vaginal mucosa. Bone mineral density declines at around 1% per month if oestrogen is not replaced. Adrenal androgen production is not GnRF sensitive and remains intact.

Although not specifically studied, GnRF vaccines are not expected to have any effect in pre-pubertal children of either sex. The phase from neonatal life to the onset of puberty is characterised by a gonadal independent suppression of hypothalamo-pituitary drive to the gonads. The pubertal transition is characterised by increasing LH secretion, implying increasing GnRF drive, and GnRF analogues are used for the suppression of the hypothalamo-pituitary gonadal axis in cases of precocious puberty (where premature elevation of oestrogen would otherwise lead to fusion of long bone epiphyseal growth plates with resultant short stature). The effects of disrupting normal puberty on bone mass accretion are less understood. Theoretically disturbing bone accretion during puberty could lead to lower final bone mass and increased risk of osteoporosis in later life if there is no catch up accretion once treatment stops. Limited evidence, however, suggests treatment of precocious puberty does not alter final bone mass.

The effects of GnRF immunoneutralisation during pregnancy are speculative. Immunoneutralisation occurring pre-implantation might be expected to interfere with maintenance of the corpus luteum and so lead to loss or termination of the pregnancy. Later in gestation, it is likely that GnRF immunoneutralisation will influence development of the fetal organs: antibodies of the IgG class do cross the placenta and would be expected to suppress the mid-gestation rise in gonadotropin secretion seen in the fetus. The placenta contains GnRF-1 and GnRF-11 but the physiological roles of these peptides have not been characterised.

Clinical experience with GnRF vaccination in humans

Clinical experience with GnRF vaccination in humans has been reported in men with prostate cancer. An experimental vaccine has been used which appears to be similar to Improvac (Aphton Co, Woodland, CA, USA). Unlike Improvac, the antigen in this case consists of native GnRF linked via a spacer to DT and is formulated in a water/oil adjuvant system. Note: oil-emulsion adjuvants would be expected to induce a stronger and longer lasting immunity than aqueous DEAE-dextran, the adjuvant system in Improvac. In twelve men with advanced prostate cancer, the subjects received either 30 or 100 µg of GnRF-DT given on three separate occasions over 6 weeks. Eleven of twelve patients developed antibodies to GnRF and a marked antibody response was seen in five subjects resulting in lowering of testosterone levels (to castrate range in four subjects) for 9 months, after which testosterone levels appeared to be on a rising plane, corresponding with declining anti-GnRF titres. Nine subjects reported injection site pain lasting up to 11 days, and two described flu'-like symptoms

on a single occasion. In a further study, twelve men were vaccinated with 3 or 15 µg doses, also on three separate occasions over 6 weeks.

Three intramuscular injections of 15 µg of antigen elicited significant antibody titres in 2/6 men and, in these two patients, serum testosterone and LH were suppressed to castrate levels. Doses of 3 µg of antigen, although they elicited a moderate anti-GnRF antibody titre in one patient, failed to reduce serum testosterone or LH in any patient. No physiological effects (decreased testosterone production) were seen after the first dose in either study.

EXPOSURE ASSESSMENT

Description of the product

Pharmaceutical form

Sterile solution for injection. Formulation is provided below:

GnRF- analogue-DT conjugate (antigen)
DEAE-dextran (adjuvant)
Thiomersal (preservative)
Water

Presentation (quantity available to the user, packaging)

Presented as a clear, colourless, water-based solution in 10, 50 and 125 dose presentations (respectively 20 ml, 100 ml and 250 ml HDPE containers), which should be stored at 2-8°C.

Method of use The product is intended for use in growing male pigs. An initial injection of 2 ml is followed at a variable interval (at least 4 weeks) by a second dose 4-6 weeks before slaughter, both administered subcutaneously in the neck. The Summary of Product Characteristics (SPC) and package leaflet recommend that a safety injector must be used.

Relevant physico-chemical characteristics

Due to the high proportion of DEAE-dextran in the product (15%), the solution is quite viscous and thus not prone to rapid surface area spreading or aerosolisation. It is also easily washed off with soap and water.

Exposure scenarios

Professional users

Professional users could conceivably come into contact with the whole product:

a) If leakage occurs during connection of the injector device to the vial

Injector devices and the containers from which they withdraw fluid are designed to be compatible with one another and, during the process of connecting a vial to an injector device, the risk of leakage is extremely low.

b) If accidental self-injection or needle stick occurs during administration to the animal

Two injections are needed for a physiological response, which is temporary in nature. On the SPC and labelling, it is stated that a safety vaccinator must be used. The Applicant will ensure that such devices are readily available on the market. As a minimum, these devices will have needle guards and a mechanism to prevent accidental operation of the trigger thereby minimising the possibility of accidental self-injection and needle stick injury.
In Australia, since 1998, similar devices have been in use and, while 3 million doses of Improvac have been sold, there has only been one case of accidental self-injection reported there. However, this

individual was not using a safety vaccinator. As at least two injections are needed for a physiological response, this individual did not suffer any undue systemic ill-effects but was advised not to use the product again. In support of user safety and in consideration that the product has been marketed outside the EU for almost 10 years, a PSUR was provided. In total three cases of self-injection had been reported. The incidence of self-injections after use of more than 7 million doses of Improvac is 0.00004%.

c) If contact occurs during disposal of product

Like all multi-dose injectable products, the Improvac vial stopper is designed to withstand multiple punctures and maintain content integrity, so the risk of leakage from used vials is extremely low. Moreover, the vial is made from polyethylene plastic and is extremely resistant to breakage.

Non-professional users

Non-professional users could conceivably come into contact with the whole product if they are involved in disposal and there is leakage. Like all multi-dose injectable products, the Improvac vial stopper is designed to withstand multiple punctures and maintain content integrity, so the risk of leakage from used vials is extremely low. Moreover, the vial is made from polyethylene plastic and is extremely resistant to breakage.

HAZARD IDENTIFICATION AND CHARACTERISATION

Relevant route of exposure	**Minimal Required Information regarding:**	
	Active ingredients	*Product as a whole, including excipients*
Oral	Not applicable	Not applicable (but overdose data in animal models available)
Dermal	Not applicable	Not applicable
Parenteral (self-injection)	Not applicable	Immunogenicity and suppression of H-P-G axis*
Ocular	Not applicable	Not applicable
Inhalation	Not applicable	Not applicable

*Hypothalamo-Pituitary-Gonadal axis

RISK CHARACTERISATION

Overdose oral data generated by the Applicant in both pigs and rats confirm that the antigen is not immunogenic by the oral route and no adverse effects are likely to occur from accidental oral exposure. Dermal or ocular contact will also not result in systemic absorption of product unless there is an open cut and sustained contact—and even then only very small quantities are likely to be absorbed. As the product is quite viscous and not prone to aerosolisation, systemic exposure via inhalation is unlikely.

Except in the case of accidental parenteral administration, the risk of the professional or non-professional user developing immunoneutralising antibodies to endogenous GnRF is very low since two systemic exposures to sufficient quantity of Improvac must occur at an appropriate (undefined) interval. Clinical experience, as described in the literature supports that, after accidental parenteral exposure, any gonadal suppression would be dose and frequency dependent, and self-limiting.

RISK MANAGEMENT

Prevention or minimisation of exposure

As previously stated, two injections are needed for an immunological and physiological response to occur (suppression of H-P-G axis), which is temporary in nature.

On the SPC and labelling, it is stated that a safety vaccinator must be used. The Applicant will ensure that such devices are readily available on the market. As a minimum, these devices will have needle guards and a mechanism to prevent accidental operation of the trigger thereby minimising the possibility of accidental self-injection and needle stick injury.

Improvac will only be available under veterinary prescription and the Applicant intends to work closely with veterinarians as part of the overall strategy for user training and risk management.

The effects of inadvertent exposure in pregnant women are difficult to predict. Pregnant women should therefore avoid exposure. On the proposed SPC and labelling, it is stated that the product should not be used by pregnant women or by those who may be pregnant.

Lastly, the label specifically states that if accidental self-injection occurs then the person should wash the injury thoroughly with clean running water, seek immediate medical attention and should not use the product, and/or any products with similar action, in the future.

Remedial action

In the event of accidental self-injection, the person is advised to seek immediate medical attention. In the case of a first injection, apart from treatment of any local soreness that might result, no remedial action is expected to be required. The person should be advised to not use the product, and/or any products with similar action, in the future. If a person presents with a possible second accidental self-injection, then remedial action may be required. Depending on the patient's sex, either testosterone or oestrogen levels in blood should be monitored. Clinically meaningful suppression of gonadal function should be managed with supportive endocrine replacement therapy (i.e. testosterone or oestrogen). In the absence of specific assays to monitor antibody titres, it is suggested that endocrine replacement therapy is continued for at least 6 months. Evidence of continued hypogonadism on stopping treatment indicates that a further period of endocrine replacement is required. Specialised fertility treatments are available in the unlikely circumstance that exposure leads to refractory gonadal suppression.
There is also evidence from autoimmune conditions that plasmapharesis results in temporary clinical improvement by lowering antibody litres.

Risk communication

The SPC and package insert adequately communicate the risk of handling/using Improvac.

B. RESIDUE ASSESSMENT

Study of residues and ecotoxicity

Introduction

The Applicant has provided extensive documentation on the risk of consumption of residues in tissues from the neck of pigs slaughtered before total resolution of injection site reactions.

The composition of Improvac is:

GnRF-analogue-DT conjugate:	Antigen
DEAE-dextran:	Adjuvant
Thiomersal:	Preservative
Water for injections:	Diluent

The CVMP previously confirmed that neither the antigen nor the adjuvant fall within the scope of Council Regulation (EC) No. 2377/90 and therefore no MRLs were required. Thiomersal is listed in Annex II of the said Regulation, and thus no MRL was required.

Diphtheria toxoid is a protein containing natural amino acids, used in WHO child diphtheria vaccination programmes. This protein is co-valently coupled to the GnRFanalogue to create the active substance, which is intended to induce antibodies against GnRF.
As the active substance is intended to produce immunity as part of an immunological veterinary medicinal product it consequently does not fall within the scope of Council Regulation (EC) No 2377/90. Studies have been provided to show that the analogue has significantly reduced biological effect compared to the native substance and that even this activity is eliminated by chemically coupling it to the carrier protein. Native peptide GnRF was previously assessed by the CVMP, as well as one synthetic analogue and it was concluded that no MRL was required. GnRF (GnRH) is included in Annex II of Council Regulation 2377/90. These previous assessments support the conclusion that no MRL is required for the active substance.

It was concluded that carcasses of pigs vaccinated with Improvac, according to label recommendations, would not contain any potentially harmful residues and that the consumption of pork from vaccinated pigs therefore poses no risk to the consumer. A zero withdrawal period was therefore proposed and accepted by the CVMP.

Food Safety Assessment of the Composition

Antigen (GnRF analogue-DT conjugate)
The antigen is a synthetic peptide analogue of GnRF conjugated to diphtheria toxoid (DT). This linkage between the GnRF analogue and DT is highly stable to physiological conditions and the GnRF analogue and the DT are therefore not likely to be disassociated from one another in the vaccinated animal.
DT is a protein of approximately 58,000 Daltons that is used in human diphtheria vaccines. It is one of the longest used (since 1930s) and safest of all human vaccines, and is used in WHO child vaccination programmes.

Since DT is a protein containing natural amino acids, the consumption of any residue by humans will expose it to digestive enzymes with subsequent rapid digestion and no toxic effect. Prior to ingestion, the process of cooking will also denature the conformation and activity of the protein.

The GnRF peptide used in the product structurally differs from the natural mammalian GnRF molecule.

Due to the very high specificity of binding of natural GnRF to receptor sites on the gonadotrope cells of the pituitary gland, this structural difference is highly significant with regard to biological recognition and response.
While the conjugate is physiologically stable, the potential hormonal activities of both the GnRF analogue and the GnRF-DT conjugate antigen have been investigated. The potential systemic bioavailability and immunogenicity of the antigen by the oral route was also investigated.

GnRF analogue and GnRF-DT conjugate antigen: Hormonal activity
A study was presented to provide evidence of the lack of hormonal activity of the analogue and the antigen conjugate (i.e., stimulatory release of luteinising hormone (LH) from the pituitary).

Study design:
As GnRF is highly conserved across mammalian species and because of extensive experience with this model, the sheep was used as the test animal. Post-pubertal female sheep were assigned to one of four groups. On day 8 of their respective oestrus cycles, when LH pulse frequency is low, the sheep were given 3 intravenous (IV) injections of morphine at half-hour intervals to suppress synthesis of endogenous GnRF. After the third morphine injection, the respective test groups were given either a single IV injection of saline, natural GnRF peptide (1 µg), the synthetic GnRF peptide analogue (50 µg), or the GnRF-DT conjugated antigen (sufficient to provide the equivalent amount of 50 µg of covalently bound GnRF peptide analogue). Baseline blood samples were obtained prior to treatment

and at 10 increasing intervals up to 180 minutes after injection. Plasma concentrations of LH were assayed by a standard radioimmunoassay with a limit of detection of 0.11 ng/ml.

Results:
The test determined that GnRF peptide analogue had a relative activity of only 0.2% compared with natural GnRF (mean GnRF analogue response ÷ mean GnRF response ÷ 50 x 100%), while the GnRF-DT antigen had no LH stimulating activity.

GnRF-DT conjugate antigen: Systemic bioavailability or immunogenicity by the oral route
Reports of two oral toxicity/bioavaliability studies, one in pigs and one in rats, were provided to demonstrate that, even at extremely high oral exposures, the antigen was not toxic nor was it systemically bioavailable or immunogenic by the oral route.

Pig study
A controlled experiment was performed to determine the antibody and hormonal response in pigs following two oral doses of Improvac. Pigs were chosen as the test animal for this study since the gastro-intestinal tract of the pig is similar to that of humans and their size allows for the simple administration of a full dose of the product.

Study design*:*
12-13 week old male pigs were randomly assigned to treated or untreated control groups. Improvac was given to the treated group in a 2 ml oral dose by mixing with a small amount of pelleted feed, prior to normal feeding. This was followed by a second oral in-feed dose 4 weeks later. Blood samples were obtained at 14, 28, and 42 days after the first oral treatment and assayed for serum testosterone and anti-GnRF antibodies. The day-42 sample was obtained 14 days after the second dose - an interval that would normally allow an anamnestic immune response to be detected if it occurred. Samples were taken between 10:00 and midday to minimise diurnal variation in testosterone levels. Serum testosterone was measured using a commercial radioimmunoassay kit and expressed as nM/L. Serum titres of antibody against GnRF were measured by a validated in-house radioimmunoassay, with a limit of quantitation (LOQ) of an antibody titre of 20. Observations on general health were also recorded.

Results/Discussion*:*
The serum anti-GnRF antibody titres for all samples were <LLOQ, following both the first and second oral doses. The absence of detectable antibodies 2 weeks post second oral dose (day 42) was particularly relevant as this is the time when high titres usually occur in pigs injected with Improvac. All pigs had measurable testosterone levels that were within normal reference ranges for animals of that age. There were no significant differences in testosterone levels between orally dosed pigs and untreated control pigs at any sampling interval. At the 42 days time point, when any effects on the immune systems would be expected to be maximal, the testosterone levels between the controls and orally administered group were not statistically different. Throughout the trial, daily observations revealed no abnormal clinical signs or adverse events in any of the pigs.

Rat study
An additional controlled study was conducted in rats to evaluate potential systemic bioavailability, immunogenic and any indirect hormonal effects of the product following oral and parenteral administration. Rats were chosen as this species is a recommended animal model to conduct oral safety studies for products administered to food producing animals that are intended for human consumption.

Study design*:*
Improvac was administered orally by gavage (PO) once on Day 1 and Day 29 to equal numbers of male and female rats per group at doses of 11.4 µg/kg, 272 µg/kg and 462 µg/kg antigen/kg (as Improvac). These doses represented approximations of potential exposure by immediate injection site consumption by a 60 kg human of multiples of 1.7, 41 and 70x, respectively. An additional two groups of equal number of males and females were given either saline or vehicle (formulation without antigen) at the same dosing volume and dosing interval. A positive control group was also included which was given 27.5 µg/kg subcutaneously (SC) also on Day 1 and Day 29.

Follow up:
Parameters for evaluation included daily clinical observations, weekly body weight, weekly food consumption and terminal haematology, coagulation, clinical chemistry and hormone analysis. Hormone analysis included LH (all animals), progesterone (females only), estradiol-17b (females only), and testosterone (males only). Rats in both the oral groups and in the SC injection positive control groups were necropsied 29 days after the second dose. Organ weights on heart, liver, kidneys, adrenal glands, pituitary and brain were obtained and tissues were collected for histological evaluation.
A satellite toxicokinetic (TK) study was conducted in parallel with the main study in which the rats were dosed either PO with 462 µg/kg or SC with 27.5 µg/kg (as Improvac). Serum anti-GnRF antibody levels and systemic bioavailability of the antigen were determined by electrochemiluminescent immunoassays (ECLIA) with a limit of quantification (LOQ) of 4.7 picomoles/ml (pmo/ml) for anti-GnRF antibodies and 1.4 pmol/ml for the product's antigen.

Results/Discussion*:* All doses were well tolerated and there were no significant changes in body weight and feed consumption. No treatment-related gross observations or histological findings were detected. There were no changes in blood biochemistry or hormonal parameters following dosing with Improvac. There were no quantifiable anti-GnRF antibody responses (LLOQ 4.7 picomoles/ml) in the sera of any of the Improvac PO dose groups. Also, the antigen was not quantifiable in the sera in any of the groups, including the SC groups.

Based on the above, the oral no observed effect level (NOEL) was thus considered to be at least 462 µg/kg, which is approximately 70x the maximum theoretical human exposure, on a weight for weight basis (based on the hypothetical scenario of consumption of an entire uncooked injection site immediately after vaccination).

Conclusion
The results from the study in sheep demonstrated that the antigenic component of Improvac was not recognised by the pituitary and had no hormonal activity. The results from the studies in pigs and rats demonstrated that the antigen was not systemically available or immunogenic by the oral route, even at highly exaggerated doses, far beyond that which a consumer could conceivably be exposed. Given the protein nature of the antigen and its consequent susceptibility to digestion, these results were not unexpected and supported a zero withdrawal period. The CVMP supported the above.

Adjuvant (DEAE-Dextran)
The antigen in Improvac is combined with aqueous diethylaminoethyl-dextran (DEAE-dextran), as the adjuvant. It is a cationic, 2-(diethylamino) ethyl ether derivative of dextran with approximately one DEAE-substituent per three glucose units and has a mean molecular weight of 500,000 Daltons. DEAE-dextran has no systemic pharmacological properties, but serves to enhance the GnRF-DT conjugate immunogenic response.
Dextran is used as a vehicle for the restoration of blood plasma volume in emergency, while DEAE-dextran has a wide range of uses including use as a vaccine adjuvant, as an agent for tranfection, as an agent for gene therapy, and as a stabiliser of proteins. DEAE-dextran is also used in human medicine as an oral treatment for hypercholesterolaemia and hyperlipaemia, where the recommended use is up to 3 grams per day.

As assurance that the use of DEAE-dextran as an adjuvant in Improvac does not present a safety hazard to consumers of edible tissue from immunised animals, two publications on long-term use of DEAE-dextran in humans were reviewed. In the first study it was demonstrated that daily oral doses of 15 g (= 250 mg/kg) in humans (which is considerably higher than the maximum theoretical dose a human could be exposed to from consuming an injection site from an animal immunised with Improvac) for two years did not produce any significant toxicological or systemic pharmacological effect. In a one-year study using daily doses of 2 to 3 g (= 50 mg/kg) in humans (which is up to 10 times higher than the maximum theoretical human exposure following consumption of an injection site of an animal treated with Improvac), no changes in the serum vitamin levels were observed.
The Applicant concluded that there were no toxicological risks from the possible consumption of DEAE-dextran residues in the meat of pigs immunised with Improvac. The CVMP concluded previously that DEAE Dextran belongs to a chemical group where no toxicological effect has been described.

Conclusion:
At the relevant dose DEAE Dextran exerts no pharmacological activity and therefore an MRL is not necessary and as such the substance is outside of the scope of the Council Regulation (EEC) No 2377/90.

Preservative (Thiomersal)
Improvac is presented in multidose containers. Thiomersal is listed in Annex II of Council Regulation (EEC) No 2377/90 (2. Organic compounds) for all food-producing species, provided that it is used only as a preservative in multi-dose vaccines at a concentration not exceeding 0.02%. Thiomersal in Improvac complies with the requirements and is therefore not subject to maximum residue limits and provides no residue concerns.

Overall conclusion
Like other immunological products, the antigen in Improvac is a protein and is readily subject to heat denaturation upon cooking and digestion upon consumption. The oral toxicity/bioavailability studies presented in the dossier demonstrated the lack of bioavailability and systemic immunogenicity of Improvac, even at highly exaggerated doses. Moreover, the antigen has been shown to be devoid of any hormonal activity. The DEAE-dextran adjuvant is considered to have no pharmacological activity at the prescribed dose and is thus outside of the scope of the Council Regulation (EEC) No 2377/90. Finally, Thiomersal used as preservative in the formulation is used for multi-dose vaccines at a concentration below the maximum of 0.02% as stated in Annex II of the aforementioned Regulation.

The proposed zero withdrawal period for Improvac is therefore justified.

OVERALL CONCLUSION ON SAFETY

A safety dossier for Improvac consisting of one consolidated study on administration of one dose, an overdose, and repeated administration of one dose was provided. Besides this, two field studies were provided in order to demonstrate the safety of the product under commercial conditions. A detailed ecotoxicity evaluation was provided as well as a detailed user and consumer safety evaluation.

Improvac does not contain either bacterial or viral antigens, live or inactivated. The product comprises a synthetic GnRF analogue conjugated to a carrier protein (diphtheria toxoid) and the conjugate is combined with an adjuvant (DEAE-dextran). After an initial subcutaneous dose of 2 ml, a second dose administered 4-6 weeks before slaughter brings about immunocastration by stimulating the production of anti-GnRF antibodies which neutralise endogenous GnRF. The gonadotrophs of the pituitary are thus no longer stimulated to release luteinizing hormone (LH), causing secondary suppression of gonadal production of testosterone and androstenone, and also a rapid depletion of skatole to castrate levels.

The CVMP previously confirmed that the adjuvant did not fall within the scope of Council Regulation (EEC) No 2377/90 at the dose contained in the product and therefore no further studies into residues or establishment of a MRL were required. Thiomersal is listed in Annex II and thus no MRL is required for the preservative of this product.

Results from the laboratory study showed that injection site reactions occurred very commonly when 8-week-old pigs were administered a single dose of Improvac. Likewise a transient rise in temperature up to 2ºC above baseline was observed, lasting for no more than 2 days. Histological examination of injection site tissues indicated persistence of inflammatory infiltration and development of fibroplasia. Injection site reactions were not recorded until resolution in all pigs (only until day 42 after single administration of one dose). Two European field studies were provided to demonstrate the safety under normal commercial conditions of pig production. There were no clear differences in pattern of evolution of rectal temperatures following injection of Improvac between vaccinates and surgically castrated pigs, as maximum increase above base level was 2ºC in both groups. Injection site reactions revealed between 60% and 85% reacting pigs, but most injection site reactions lasted no more than 2 days. A number of pigs were recorded with injection site reactions at slaughter (the number would be recorded as common in the adverse reactions terminology). Significant differences between sites were

recorded in the safety results. Growth performance was not adversely affected by Improvac administration.

With regard to consumer safety, none of the components of Improvac have oral activity and the consumption of meat from vaccinated animals, including injection site tissue, is not considered to be harmful. In terms of ecotoxicity, there are no excreted metabolites from Improvac.

A risk assessment for user safety was carried out. Self-injection by accident has potentially serious endocrinological implications, and the Applicant has made specific warnings and instructions for the administration of the product in the SPC to this effect.

Overall the safety profile of the product was considered acceptable by the CVMP.

4. EFFICACY ASSESSMENT

INTRODUCTION

Several chemical substances are responsible for causing boar taint, of which androstenone and skatole are regarded as the most important (Hansson and others, 1980; Dijksterhuis and others, 2000). To those individuals sensitive to androstenone, the smell resembles urine or sweat, with onion-like, ammonia-like, faecal and musk-like components. Skatole has a distinctive faecal-like odour. Other chemicals thought to contribute to boar taint include androstenols, metabolites of androstenone (Brennan and others, 1986; Brooks and Pearson, 1989), and indole (Garcia-Regueiro and Diaz, 1989, Moss and others, 1993; Annor-Frempong and others, 1997a; Ruis and Garcia-Regueiro, 2001), but they seem to be of less importance because of their relatively weak odour and different lipophilic properties. Androstenone acts as a boar pheromone by inducing standing posiure of the sow during mating. This pheromone therefore is of essential importance in the swine reproductive physiology. A major European study sampling 4,313 entire male carcasses and 223 gilt carcasses from pigs reared under normal commercial conditions showed that despite significant national differences in liking for meat from entire males, the degree of dislike increases as levels of both androstenone and skatole increases (Bonneau and others, 2000; Matthews and others, 2000).

Female consumers are known to be more sensitive to boar taint than males (Matthews and others, 2000) Although a male bias exists for the inability to detect androstenone (Gilbert and Wysocki, 1987; Baydar and others, 1993), it is thought that true androstenone anosmia in the human population is actually uncommon and occurs in only 1.8-6.0% of young healthy adults (Bremner and others, 2003). Individuals highly sensitive to androstenone odour react to both androstenone and skatole, whereas individuals with mild androstenone sensitivity react to skatole only (Weiler and others, 2000). Skatole and androstenone levels in fat are significantly correlated with scores produced by sensory taste panels for skatole odour, but not with scores for androstenone odour alone.

Hypothalamic GnRF (designated GnRF1 or mammalian GnRF) is one of four decapeptide isoforms found in mammals and is the prime regulator of reproductive function. After secretion into the hypophyseal portal circulation, it binds with and activates GnRF1 receptor sites expressed on the surface of the pituitary gonadotrope cells. The resulting release of the gonadotrophic hormones FSH (follicle-stimulating hormone) and LH (luteinising hormone) results in the stimulation of the steroidogenic and gametogenic functions of the gonads of both sexes (for review see: Neill, 2002). In the male, luteinising hormone stimulates the Leydig cells in the interstitial testicular tissue to produce androgens, including testosterone and androstenone. The boar is unique among mammals in that it specifically produces high quantities of androstenone, the output from the mature testes being some ten times that of testosterone (Claus and others, 1971). Furthermore, in contrast to other mammals, the boar possesses relatively large amounts of testicular interstitial tissue (Fawcett and others, 1973) of which 70% is composed of Leydig cells, each cell possessing approximately 35.000 LH-binding sites (Peyrat and others, 1981). Thus the potential output of androstenone in the post-pubertal boar is exceptionally high.

In male pigs, the levels of androstenone and skatole can be correlated (Bonneau and others, 1992; Annor-Frempong and others, 1997b). Skatole levels in fat are related to hepatic metabolism (Friis, 1995: Squires and Lundstrom 1997; Babol and others, 1998a; Babol and others, 1998b). Elevated levels of sex steroids reduce hepatic metabolism of skatole and subsequent clearance from the body, resulting in increased accumulation in fat tissue (Babol and others, 1999). A significant reduction in androstenone output from the testes would therefore be expected to reduce skatole levels in fat. The results of studies presented by the Applicant have demonstrated evidence of this effect. The threshold at EU level for both components to define the acceptability of a carcass with regard to boar taint was discussed. On the basis of a comprehensive analysis of the many different publications on the subject the Applicant proposed that the upper individual threshold for androstenone is 1.0 µg/g and upper individual threshold for skatole is 0.22 µg/g. For the worst case presentation i.e., fresh unprocessed pork, combined lower limit consumer thresholds of 0.5 µg/g androstenone and 0.2 µg/g skatole in fat

(which, for one compound or another, are in current use by the pork industry in several EU Member States today) would appear to be conservative.

Skatole (μg/g)	Androstenone (μg/g)		
	Low <0.5	Medium 0.5-1.0	High >1.0
Low <0.1	Low risk	Low risk	Medium risk
Medium 0.1-0.22	Low risk	Medium risk	High risk
High >0.22	Medium risk	High risk	High risk

The CVMP concluded favourably on the cut-off values, and is in favour of the conservative, consumer-friendly approach for androstenone (0.5μg/g fat). As presented above a low risk for tainted meat could be obtained either with a medium androstenone level (0.5-1.0 μg/g fat) combined with a low skatole level (<0.1μg/g fat) or with a low level of androstenone (<0.5 μg/g fat) combined with a medium level of skatole (0.1-0.22 μg/g fat). A medium risk would occur when both biomarkers for boar taint occur in medium levels (androstenone 0.5-1.0 μg/g fat; skatole 0.1-0.22 μg/g fat). When both biomarkers exceed these cut off values at the same time it would imply a high risk of the meat to be regarded as tainted.

GENERAL REQUIREMENTS

The pivotal studies were carried out in accordance to the requirements stated in Annex I, Part 8 of Directive 2001/82/EC as amended by Directive 2004/28/EC, the Ph.Eur. General Text Section 5.2.7. and VICH GL9 on Good Clinical Practice. All studies were conducted in the appropriate category of the target animal species, e.g. entire, male fattening pigs. In order to confirm the effects of vaccination, it was necessary to include both castrated pigs to demonstrate results comparable to surgical castration, and entire boars, to compare the Improvac group with animals with normal androgen functions. Vaccination was carried out by the recommended route using the proposed schedule of administration.

The main aim of the presented studies was to demonstrate suppression of androgenicity. The pivotal studies included one recent GLP Minimum Potency Laboratory Trial and four recent field trials under commercial EU-conditions, which are tabularised and commented on below. Non-pivotal studies are included in this assessment report in a summarised form and are considered supportive evidence.

The serum concentrations of specific anti-GnRF antibodies and inhibition of serum testosterone were the primary indicators of the immunological effect induced by Improvac and were measured several times before slaughter. This was not possible for the key boar taint compounds, which could only be measured after slaughter when belly fat samples were analysed. The efficacy claim is therefore not to prove a reduction of the concentrations of boar taint compounds, but to demonstrate a low incidence of pigs with skatole and androstenone levels in fat tissue above certain thresholds, where the risk of boar taint is substantial. The efficacy criteria proposed for the control of boar taint is the control of skatole and androstenone below a threshold of 0.22 μg/g tissue and 1.0 μg/g tissue, respectively. No standard upper limits are currently established in the EU.

LABORATORY TRIALS

The recommended dose (minimum antigen content: 300 μg/dose) was established in two studies, which are considered supportive in this dossier:

- Controlled Dose Determination Study,
- Dose Justification Study

Another supportive study investigates onset and duration of immunity and is summarised below:

- Window of Suppression Trial,

A recent pivotal laboratory efficacy study using a batch with minimum potency was conducted under controlled GLP conditions, including untreated control animals as required in Directive 2001/82/EC:

- Efficacy of Minimum Potency Improvac in Male Pigs

Determination of the Dose.

Study design

This GLP compliant study included entire male pigs from 14 – 15 weeks old at Day 0.
7 groups were immunised with a 2 ml dose containing either 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg, 1.2 mg or 2.0 mg of active conjugate. One group was placebo and received 2 ml of only adjuvant and carrier. The pigs received one dose at Day 0 and another one 4 weeks later. Four weeks following the second vaccination pigs were slaughtered.

Follow-up

Examination for and scoring of site reactions took place at 7, 14, 21 and 28 days after both doses. At slaughter, any residual reaction was dissected out and stored in formalin for possible histological examination. Serum samples were collected at 2 weeks post second dose and prior to slaughter for anti-GnRF titres and testosterone concentration measuring. Tissue samples at slaughter: Tissues from both testes were collected and weighed, and a 2x5x5 cm fat sample from the belly was collected for androstenone and skatole analysis.

Results

a) Anti-GnRF titres (range) two and four weeks after boost vaccination

There was no evidence of a dose-response-effect. At 4 weeks, titres were generally 3-4 fold lower than the median response at two weeks after the 2nd vaccination.

b) Testosterone concentrations two and four weeks after 2nd vaccination (nM/L)

An effective dose was expected to result in serum testosterone levels of <2nM 2 weeks after the 2nd vaccination and <5nM after 4 weeks. At two weeks post vaccination, all treated pigs but three had low testosterone levels <2nM, compared to 50% in the control group. At 4 weeks post vaccination 87.5%treated pigs still had low testosterone levels <2nM, as had 25% of controls. Treated pigs with testosterone>2nM were seen in several groups. When data from lower dose groups (0.2-0.6 mg) were compared with higher dose groups (0.8-2.0), there was a tendency for lower doses to be more effective in suppressing testosterone production.

c) Paired testes weight at slaughter (g)

Testes weight was generally lower in vaccinated pigs than in placebo animals ($p<0.5$). No significant differences between treatment groups were seen. The animals with relatively large testes often also had low anti GnRF-titres and high serum testosterone levels.

Conclusions:

There was no evidence of a dose-response-effect. The CVMP considered the results from this non-pivotal study supportive. The Applicant stated that as the lower Improvac doses appeared to be equally effective, it was decided to base future work on a dose of 0.4 mg of active substance. It was demonstrated that none of the other developmental combinations were superior to the final selected Improvac formulation.

Onset and Duration of Immunity

Onset and duration of immunity was first investigated in a GCP compliant study and later was confirmed in other laboratory and field studies. Due to the nature of Improvac, not being a classical immunological medicinal product intended to protect against a pathogenic organism, challenge studies were not relevant.
After the first vaccination, a non-specific, IgM mediated, non-specific priming of the immune system is induced. A specific immunological effect due to the production of IgG anti-GnRF antibodies is only apparent after the second dose, administered at least 4 weeks after the first vaccination. Antibody titres peak within 1-2 weeks and decline thereafter. Likewise, six weeks after the 2nd dose serum testosterone levels rise above the lowest level of quantification. The achieved suppression of androgenic functions is long enough to result in significantly reduced testicular development in vaccinated animals. Less consistently, the proportion of vaccinated animals with low or undetectable

androstenone- and skatole concentrations in belly fat samples is comparable to surgically castrated control pigs. This effect is not significant before 4 weeks after the completion of vaccination, and the vaccination schedule must therefore be completed at least 4 weeks before slaughter.

Study Title: Window of Suppression Trial
This GLP compliant study used entire male pigs. They were 14 – 15 weeks of age at Day 0 (day of the first vaccination). The animals were divided into 7 groups and received 2 ml of Improvac or placebo at day 0 and another one 4 weeks later. The target conjugate content of the batch used was 200 µg/ml.

Follow-up
Blood samples at 2nd vaccination and slaughter (anti-GnFR titres and serum testosterone levels) were collected. Body fat samples for skatole and androstenone analysis were also collected. Testes size prior to slaughter, and testes weight and length of the bulbo-urethral gland at slaughter were determined.

Conclusions:
Optimal suppression of boar taint substances in the fat of vaccinated pigs occured 4-6 weeks after administration of the 2nd dose of Improvac.The results from this non-pivotal study were considered to be only supportive.The window of suppression was small. The effect on testes size and boar taint substances was not significant before 4 weeks after the second dose. The duration of this effect was less clearly defined.

On the basis of this study the Applicant revised section 4.2 of the SPC to provide a clear statement on onset and duration of immunity as well as information in section 4.5 on minimum time to slaughter and guidance if pigs cannot be slaughtered within the recommended window.

The Applicant provided an additional GCP compliant study to clarify the duration of Immunity.

Study Title: Efficacy of Boar Taint Control Ten Weeks After the Second of Two Doses of Minimum Potency Improvac Given Four Weeks Apart

Animals were assigned to one of two treatment groups one week before the study:

	Dosage	Regimen	Route of administration
T01	2x2 ml	Saline	s.c. injection
T02	2x2 ml	Improvac vaccination	s.c. injection

Treatment
A subcutaneous immunisation was given at the base of the ear with 2 ml of a minimum potency batch of Improvac on D0 and D28.

Follow-up
The following were measured:
- anti-GnRF antibodies and serum testosterone after vaccination
- androstenone, skatole and indole in belly fat at slaughter. Bulbourethral gland dimensions.

Results
A. Anti-GnRF antibodies
The proportion of vaccinated pigs with anti-GnRF levels below the level of quantification increased from 1.1% 28 days after the 2nd vaccination, to 23% at slaughter.. At both timepoints, the proportion was significantly lower than in the placebo.The geometric mean of antibody titres of pigs in groups T01 and T02 were similar at the time of first vaccination. Twenty-eight days after second vaccination, the geometric mean titre of pigs with quantifiable titres in group T02 (160 U/mL) was five times higher than in entire boars (33.1 U/mL), and almost two times higher at slaughter, ten weeks after 2nd vaccination. These differences were significant at both timepoints.

B. Testosterone levels Lowest Limit of Quantification (LLOQ) = 0.1 ng/ml
Twenty-eight (28) days after the second vaccination, the proportion of pigs with a testosterone concentration <0.1 ng/mL had fallen two thirds in entire males (14%) and had more than doubled (77%) in vaccinated pigs. By the time of slaughter, no entire pigs and only 5% of vaccinated pigs had serum testosterone concentrations <0.1 ng/mL. Differences in the proportions of pigs with testosterone concentrations below 0.1 ng/mL were significantly different 28 days after second vaccination and at slaughter.
The geometric mean of serum testosterone level at slaughter had increased to about 40% of the mean level among entire boars. Differences between geometric mean titres of pigs with quantifiable testosterone concentrations were statistically significant at slaughter.

C. Androstenone, skatole and indole
The Lowest Limits of Quantification (LLOQ) were 200 ng/g, 33.9 ng/g and 23.9 ng/g for androstenone, skatole and indole, respectively. Only 27% of entire boars (T01) compared with 84% vaccinated pigs (T02) had androstenone concentrations below the level of quantification, a difference which was significant. No significant differences were seen between treatment groups in terms of the percentage of pigs with skatole concentration levels below the level of quantification. Ninety-eight per cent (84/86) of entire boars (T01) compared with 93% of vaccinated pigs (T02) had levels of indole in belly fat below the level of quantification – a difference which was not significant.

There was a statistically significant difference in the proportion of pigs in pens with a concentration of androstenone <500 ng/g between entire boars (60%) and pigs vaccinated with Improvac (97%). There was also a statistically significant difference in the proportion of pigs in pens with a concentration of androstenone <1,000 ng/g between entire boars (87%) and pigs vaccinated with Improvac (100%). The geometric androstenone mean among vaccinated pigs with quantifiable androstenone levels was 417ng/g.

Bulbourethral glands
The bulbourethral glands of pigs vaccinated with Improvac (T02) were smaller and more variable in size compared with those of entire boars (T01).

Conclusions:
Overall this study demonstrated that vaccination significantly increased the proportion of pigs having androstenone levels below the level of quantification (200 ng/g) at the time of slaughter, approximately 10 weeks after the second dose, when compared with unvaccinated entire boars (83.5% of vaccinates compared to 26.7% of controls). Therefore duration of immunity of 10 weeks was considered reasonable, as following this period the levels of testosterone return to normal.

IV.C.3 Pivotal Laboratory Efficacy Trial
Study Title: Efficacy of Minimum Potency Improvac in male pigs
Study design
In this GCP-compliant study pigs were between 13 and 15 weeks on Day 0. A number of these animals received Improvac twice with an interval of 28 days and a similar number of pigs received saline. Four weeks after the second immunisation all animals were slaughtered (Day 56).
The batch used was close to the target concentration.

Follow-up
i) Physical examination D0 and D28, general health daily.
ii) Blood sampling: D0 and D55 for antibodies against GnRF and testosterone in serum
iii) Belly Fat sample: D56 (at slaughter) for androstenone, skatole and indole in belly fat
Results
A. Anti-GnRF antibodies
All pigs had quantifiable titres at D0 and D55. At day 55, vaccinated pigs had a geometric mean anti-GnRF titre that was significantly higher than in control animals (214 U/ml vs. 23.0 U/ml).

B. Testosterone
At day 0, the proportion of pigs with testosterone levels below the level of quantification was comparable in vaccinated and control pigs. So were the quantifiable levels of testosterone measured in the two groups. At day 55, 85 % of vaccinated pigs still had a testosterone level BLOQ, compared with only 1% of control pigs; and the geometric mean level of testosterone was now significantly lower among vaccinated pigs than among controls.

C. Androstenone, skatole and indole
The LLOQ were 200 ng/g, 13.2 ng/g and 12.9 ng/g for androstenone, skatole and indole, respectively.
For pigs with concentrations above the LLOQ there was no significant difference between geometric mean concentrations of taint compounds in belly fat samples from vaccinated and control pigs, respectively. However, androstenone levels were below the LLOQ in 97% of vaccinated pigs and in only 8% of the controls. Both groups had low-level findings of skatole at slaughter.
There was a statistically significant difference in the proportion of pigs with low androstenone levels (<500 ng/g) between entire boars (29%) and vaccinated pigs (98%). There was also a statistically significant difference in the proportion of pigs with low and medium (<1000 ng/g) androstenone levels between entire boars (66%) and vaccinated pigs (100%).

Conclusions:
The results for the distribution of the boar compounds skatole and androstenone were related to the proposed threshold levels. All vaccinated pigs were well below the threshold for androstenone and none of the pigs were above the threshold for skatole, the latter perhaps reflecting the prevailing conditions of husbandry. Overall this study demonstrated that vaccination with Improvac according to the recommended schedule using a minimum-potency batch of the product stimulates the production of anti-GnRF antibodies with consequential reduction in testosterone and androstenone concentrations at the time of slaughter four weeks after the second dose.

FIELD TRIALS
Five field studies were presented in detail. Four pivotal field studies under typical European commercial conditions of pig production were conducted in Spain, Germany, Denmark and Hungary, as required by Directive 2001/82/EC. A fifth study including heavier pigs was conducted in Italy.
Supplementary efficacy data were provided from a controlled study carried out in Brazil, and various bibliographic references.

1) Study Title: To demonstrate the safety and efficacy of Improvac in controlling boar taint in heavy male pigs under commercial field conditions in Italy
Three groups of pigs were included and were divided into 3 groups. One group (T01) was surgically castrated, another (T02) received 3 doses of Improvac and the third (T03) received two doses of Improvac. Immunisation was conducted at 10 - 11 weeks, 26 - 27 weeks and 36 - 37 weeks for T02 and 10-11 weeks and 26-27 weeks for T03. Blood samples were collected at each vaccination, 14 days after the third vaccination and at slaughter (for T02: 6 weeks after last vaccination, for T03: 16 weeks after last vaccination).

Results
Three vaccinations (T02) resulted in a substantial rise in circulating antibodies against GnRF following the 3rd injection. Among pigs with quantifiable titres at slaughter six weeks after the 3rd treatment, the titre had declined to a mean of 224 U/ml compared with 82 U/ml among castrates and 70.1 U/ml among pigs vaccinated twice (T02). The testosterone level among most T02 pigs at slaughter was close to the LLOQ (71% <LLOQ) and comparable to the levels among castrated pigs (91%<LLOQ). T03 pigs differed from the other two groups. After a decline in mean testosterone concentrations following the 2nd vaccination, 87% pigs exhibited an increase of testosterone levels before slaughter, arriving at a mean value being higher at the time of slaughter than before the 2nd injection 2.36 ng/ml. It was shown that 2% of the castrated pigs (T01) had a belly fat androstenone concentration above the proposed taint threshold for androstenone of 1,000 ng/g, and that 8% of the pigs receiving two doses of Improvac (T03) had an androstenone concentration above this level, when

slaughtered 16 weeks after the second dose. Using a more conservative threshold limit of 500 ng/g for androstenone, the corresponding percentages were 2% and 16% for groups T01 and T03 respectively. In contrast, none of the pigs given three doses of Improvac (T02) had a belly fat concentration of androstenone above 1,000 ng/g or even 500 ng/g. No pig had belly fat skatole levels above the lowest proposed threshold for taint (200 ng/g). There was no significant treatment effect on body weight.
Sixteen weeks after administration of the second dose and in heavy pigs weighing as much as 160-200 kg, control of boar taint was not reliable with a few pigs regaining at least partial testicular function and showing boar taint. Nevertheless, administration of a third dose six weeks before slaughter was sufficient to safely restore reliable taint control.

Conclusions:
From this study the CVMP concluded that the recommended use is to slaughter 4 to 6 weeks after the second dose while sending pigs for slaughter more than 10 weeks after the second dose should only be an exceptional occurrence. A specific administration of a third dose in the SPC was not deemed appropriate and thus a recommendation for a third vaccination was not included in the SPC.

2) Study Title: To confirm the Safety and Efficacy of Improvac Vaccine in Controlling Boar Taint in Male Pigs under Commercial Field Conditions in Spain
Animals were randomly assigned to one of three treatment groups as follows:

	Dosage	Regimen	Route of administration
T01	-	Surgical castration	-
T02	2x2 ml	Improvac vaccination	s.c. injection
T03	-	Entire boars	-

Animals were given a subcutaneous injection of 2 ml of a minimum potency batch, twice with 4 and 5.5 weeks interval (site 1 and 2, respectively). The 2nd vaccination was given 4.5 weeks prior to slaughter at both sites. All pigs were weighed at a few days of age and physically examined. Surgically castrated and vaccinated pigs were followed more closely around castration, weaning, and vaccination.

Follow-up
Efficacy was assessed by
-Measuring anti-GnRF antibodies, changes in testicular size and serum testosterone after vaccination
-Measuring androstenone, skatole and indole in belly fat at slaughter.
Impact of surgical castration vs. vaccination was followed by monitoring body weight, clinical abnormalities and carcass quality at slaughter.
Safety was also assessed in this study; these issues are discussed in part 3.

Results:
A. Anti-GnRF antibodies

Treatment	**Percentage of pigs with anti-GnRF antibody titres BLOQ**			
	Vaccination 1	Vaccination 2	Vacc.2 +14D	Slaughter - 1D
T01	85.8	82.3	73.8	80.7
T02	90.9	**19.3**	**4.2**	**0.5**
Treatment	**Geometric mean titres of quantifiable anti-GnRF antibody** (U/ml),			
	Vaccination 1	Vaccination 2	Vacc.2 +14D	Slaughter - 1D
T01	24.2	27.8	31.1	24.5
T02	27.2	44.3	**568**	**255**

Fifteen to twenty percent of castrated pigs had detectable levels of anti-GnRF antibodies, but the titres remained low and were probably attributable to an unspecific background reaction during the ELISA test. Proportions of pigs in T01 and T02 with quantifiable antibody titres were statistically different at the time of 2nd vaccination, 14 days later and at slaughter, with a marked fall in vaccinated pigs without detectable antibody response between the 1st and 2nd vaccination. Correspondingly, the mean anti-GnRF antibody levels in vaccinated pigs increased clearly after the 1st vaccination compared with castrated pigs, the highest level being measured 14 days after the 2nd vaccination. At the time of slaughter, the values seem to decrease again.

B. Testicular measurements
Between the 1st vaccination and slaughter, the testicles grew approximately 23 mm and 8 mm in length and width, respectively. No difference was apparent between vaccinated pigs from the two trial sites, or left and right testicles.

C. Testosterone levels
Amongst surgically castrated pigs, only traces of testosterone were found in serum samples. In the vaccinated group, levels dropped BLOQ in 93% and 80% of pigs 14 days after the 2nd vaccination, and at slaughter, respectively. The geometric mean testosterone level also fell significantly in this time period.

D. Androstenone, skatole and indole
The LLOQ were 200 ng/g, 20 ng/g and 7.05 ng/g for androstenone, skatole and indole, respectively.

Androstenone levels were BLOQ in T01 and T02 pigs, while 61% of entire boars (T03) had quantifiable androstenone levels in belly fat samples.
For both skatole and indole, quantifiable levels in belly fat at slaughter were not significantly different in groups T01 and T02. Both skatole and indole quantifiable levels were higher in T01 and T02 animals from site 2 than from site 1, when BLOQ value were substituted with LLOQ/2 and geometric least square means for groups T01 and T02 from site 2 were compared with those from site 1.

Distribution of pigs by belly fat concentration of androstenone and skatole
All castrated and vaccinated animals had low androstenone levels in belly fat samples, while 1/3 of the boars had medium or high levels. The skatole concentrations in belly fat samples from castrated and vaccinated pigs were also less frequently medium or high compared to samples from entire boars.

E. Carcass evaluation
The SEUROP scoring system was used. Pigs from site 2 achieve lower grades at slaughter than pigs from site 1, their size seemed more variable, and the best grade was U, which is the third best grade. By contrast, pigs from site 1 are mainly graded E, and some carcass weights were between 50 and 60 kgs.

F. Bodyweight and Average Daily Gain (ADG)
At castration and weaning, bodyweights of pigs in groups T01 and T02 were comparable.
ADG from castration to weaning and from castration to slaughter, respectively, was comparable in both treatment groups.

<u>Conclusions</u>
Anti-GnRF antibody titres and testosterone concentrations:
There were significant treatment group differences in the percentage of animals with quantifiable anti-GnRF antibody titres and testosterone levels, at each timepoint and across sites. Comparable geometric means were observed at both sites. Results for androstenone levels in belly fat (BLOQ), by site, showed no significant differences between surgical castrates (T01) and vaccinated pigs (T02) at either site.
Although minor differences existed between sites with regard to some of the parameters, it also appeared that the effect of the product was comparable at site A and B, respectively. The suppression

of testicular function is evident among vaccinated pigs, although testosterone concentrations seemed to increase again at the time of slaughter, which was correlated with a decrease of GnRF antibody titres. However, the level of boar taint compounds still was low at this time point and at both sites.

3) Study Title: Evaluation of the Safety and Efficacy of Improvac Vaccine in Controlling Boar Taint in Male Pigs under Commercial Field Conditions in Germany
This was a GCP compliant study which took place in two sites.
In site A: pigs aged 19-20 weeks at 1st vaccination were included and in site B: pigs aged 19-21 weeks at 1st vaccination were included

In each site animals were assigned to one of three treatment groups:

	Dosage	Regimen	Route of administration
T01	-	Surgical castration	-
T02	2x2 ml	Improvac vaccination	s.c. injection
T03	-	Entire boars	-

Treatment
A subcutaneous injection of 2 ml from a minimum potency batch was given twice with 4 weeks interval in T02 animals. The 2nd vaccination was given 4 or 5 weeks (site A) and 4.5 weeks (site B) prior to slaughter, respectively. All pigs were weighed at a few days of age and physically examined. Surgically castrated and vaccinated pigs were followed more closely around castration, weaning, and vaccination and blood samples were collected, testes and body weight were measured.

Follow-up
Efficacy was assessed by
-Measuring anti-GnRF antibodies, changes in testicular size and serum testosterone after vaccination
-Measuring androstenone, skatole and indole in belly fat at slaughter.
Impact of surgical castration vs. vaccination was followed by monitoring body weight, clinical abnormalities and carcass quality at slaughter.
Safety issues are discussed in Part 3.

Results
A. Anti-GnRF antibodies
Proportions of pigs in T01 and T02 with quantifiable antibody titres were statistically different at the time of 2nd vaccination, 14 days later and at slaughter, with a marked increase in vaccinated pigs with detectable antibody response between the 1st and 2nd vaccination. The highest anti-GnRF antibody levels were measured 14 days after the 2nd vaccination. At the time of slaughter, the titres declined again (> 50%).

B. Testicular measurements
Between the 1st vaccination and slaughter, in vaccinated pigs the testicles grew approximately 8 mm and 6 mm in length and width, respectively. By contrast, mean testicle size in entire boars increased 35 mm in length and 20 mm in width during this period. No difference was apparent between pigs from the two trial sites, or left and right testicles.

C. Testosterone levels
The proportions of pigs with quantifiable serum testosterone levels were statistically different in castrated and immunised groups at 1st and 2nd vaccination, 14 days after the 2nd vaccination and at slaughter.

D. Androstenone, skatole and indole
The LLOQ were 200 ng/g, 20 ng/g and 7.05 ng/g for androstenone, skatole and indole, respectively.
Except for one pig in each group, androstenone levels were BLOQ in T01 and T02 pigs, while 59% of entire boars (T03) had quantifiable androstenone levels in belly fat samples.

Among pigs with quantifiable levels of skatole in belly fat at slaughter there was no significant difference in skatole between groups T01 and T02. Among pigs with quantifiable results, mean skatole levels apparently were higher in entire boars, but the proportion of animals with values >LLOQ was smaller (71%) than in the other two groups.
Skatole quantifiable levels were higher in T01 and T02 animals from site A (43.5, 48.8 ng/g respectively) than from site B (26.9, 31.5 ng/g respectively) when BLOQ values were substituted with LLOQ/2, and geometric least square means for groups T01 and T02 from site 2 and were compared with those from site 1.
Except for one or two pigs from each group, all belly fat samples contained quantifiable levels of indole. No significant differences were evident between groups T01 and T02 or site A and B.

Distribution of pigs by belly fat concentration of androstenone and skatole
One entire boar had high levels of skatole in belly fat, combined with medium androstenone concentrations. Medium skatole concentrations were detected in few (2.7-5.9%) animals from each treatment group together with low androstenone values. The majority of pigs could be classified as having low levels of skatole. No significant differences were detected between groups.

E. Carcass evaluation
The SEUROP Grade Scoring system was used. Lean meat percentages were highest in boars, followed by Improvac treated pigs, then castrated pigs. No carcasses were graded the highest grading, S, or the lowest grading, P.

E.2. Carcass Weight and Fat Thickness
No significant difference was detected between groups T01 and T02, but mean hot carcass weights of pigs from site B were heavier than those from site A. The least square mean backfat thickness was significantly higher in castrated pigs compared with vaccinated pigs ($p=0.0021$). Boars had slightly lower mean values than vaccinated pigs.

E.3. Other Carcass Characteristics
No significant differences were detected between groups T01 and T02, except in the percentage of muscle in the belly. There also was a less significant difference between castrated pigs from site A vs. site B, where the % of muscle in the belly was 6% higher in pigs from site A.

F. Bodyweight and Average Daily Gain (ADG)
At castration and weaning, bodyweights of pigs in groups T01 and T02 were comparable. At weaning, the mean bodyweight of T03 animals was smaller compared to T01 and T02. The Average Daily Gain (ADG) from castration to weaning and from castration to slaughter, respectively, was comparable in both treatment groups.

Conclusions:
No obvious contrasting trends are evident at the different trial sites. Although minor site-related differences existed for some of the parameters, it also appeared that the effect of Improvac was comparable at both sites. The suppression of testicular function was evident among vaccinated pigs, although testosterone concentrations seemed to increase again at the time of slaughter, which is correlated with a decrease of GnRF antibody titres. However, the level of boar taint compounds still was low at this time point and at both sites. Similarly, the results from carcass quality measurements were comparable between sites.

4) To Evaluate the Efficacy and Economic Benefits of Improvac Vaccine in Male Pigs raised under Commercial Field Conditions in Denmark

The study was GCP compliant. Animals were assigned to 3 treatment groups at the age between 2 – 5 days, as follows:

T01: included Improvac vaccinated pigs (they were vaccinated twice with 4 weeks interval. They were 11 -12 weeks old at first vaccination. The second vaccination was 36 days prior to slaughter and animals were 16 – 17 weeks old.

T03: included entire boars.

The antigen content of the batch used for vaccination was at the minimum

The Applicant provided clarifications as to why in Denmark and Hungary the field trials are conducted in one site, where in Spain and Germany in two sites. The Applicant clarified that in Spain and Germany, the Applicant was able to identify two sites in each country that had suitable husbandry and management practices to allow a small number of entire boars to be raised alongside the castrated and vaccinated pigs, and also that had access to a slaughter house that would be able to accept the vaccinated pigs and to take the samples at time of slaughter. In Denmark and Hungary, however, the Applicant was able to identify only one site at the time of initiation of the efficacy studies that met the required conditions, in particular access to a slaughter house that could accept the vaccinated pigs and take the samples at time of slaughter. This was considered acceptable.

Follow-up

Efficacy was assessed by

-Measuring anti-GnRF antibodies, changes in testicular size and serum testosterone after vaccination

-Measuring androstenone, skatole and indole in belly fat at slaughter.

Impact of surgical castration vs. vaccination was followed by monitoring body weight, clinical abnormalities and carcass quality at slaughter.

Safety issues are discussed in Part 3.

Results

A. Anti-GnRF antibodies

Treatment	**Percentage of pigs with anti-GnRF antibody titres BLOQ**			
	Vaccination 1	Vaccination 2	Vacc.2 +14D	Slaughter -1D
T01	90	87.5	69.4	80
T02	97.1	**25.0**	**0**	**1.7**
T03	100	**-**	**-**	100

Treatment	**Geometric mean titres of quantifiable anti-GnRF antibody** (U/ml),			
	Vaccination 1	Vaccination 2	Vacc.2 +14D	Slaughter -1D
T01	26.5	22	22.5	58.7
T02	21.6	44.1	**669**	**180**

Some castrated pigs also had anti-GnRF antibody levels >LLOQ, which were considered to be due to unspecific binding during the ELISA test. Proportions of pigs in T01 and T02 with quantifiable antibody titres were statistically different at the time of 2nd vaccination, 14 days later and at slaughter, with a marked increase in vaccinated pigs with detectable antibody response between the 1st and 2nd vaccination. The highest anti-GnRF antibody levels were measured 14 days after the 2nd vaccination. At the time of slaughter, the titres decline markedly. None of the boars had quantifiable levels of antibodies against GnRF.

B. Testicular measurements

No difference was apparent between pigs from the two treatment groups at the time of 1st vaccination, for left and right testicles. At slaughter, differences in size and weight between vaccinated pigs and entire boars were pronounced.

C. Testosterone levels LLOQ= 0.1 ng/ml
At first vaccination there were no significant differences between the two treatment groups (T02 and T03). At slaughter however, the differences were significant, and all of the vaccinated pigs had testosterone below the level of quantification.

D. Androstenone, skatole and indole
The LLOQ were 200 ng/g, 13.2 ng/g and 12.9 ng/g for androstenone, skatole and indole, respectively.

Taint Compounds	**Geometric Mean Concentrations (ng/g) in belly fat of pigs with levels above LLOQ**		
	T01	T02	T03
Androstenone	0	514	**966**
Skatole	34.3	40.7	87.2
Indole	17.8	16.8	32.2

Except for one pig in T02, androstenone levels were BLOQ in T01 and T02 pigs, while all entire boars (T03) had quantifiable androstenone levels in belly fat samples. Almost all pigs regardless of treatment had detectable levels of skatole in the belly fat samples at slaughter. Quantifiable skatole levels were not significantly different in groups T01 and T02. Belly fat samples from 39 % of castrated pigs, 75 % of vaccinated pigs and 75 % of boars, respectively, contained quantifiable levels of indole. No significant differences were evident between groups T01 and T02, where the mean indole values seemed to be about half the mean of values among entire boars.

Distribution of pigs by belly fat concentration of androstenone and skatole

Androstenone levels >1000 ng/g belly fat were only measured in entire boars, and five of those also had high levels of skatole. Only 21.4 % had androstenone concentrations <500 ng/g, compared to 98.4 % of animals in groups T01 and T02. In relation to skatole no significant differenes were detected between Improvac treated animals and surgical castrates. Both Improvac treated animals and surgical castrates had significantly lower skatole concentration in belly fat compared with entire boars. All castrated and vaccinated pigs except for one in each group could be classified as having low levels of both compounds. No significant differences were detected between groups.

E. Carcass evaluation
There were no statistically significant differences in meat percentage, ham meat-, middle meat- and front meat percentages and prepared carcass weights between castrated and vaccinated animals.

F. Feed consumption and Conversion
No statistical analysis was performed on these variables due to the low number of pens and inadequate statistical power. Feed conversion ratio was numerically lower in the vaccinated pigs, compared to the barrows, particularly in the final part of the fattening period.

G. Bodyweight and Average Daily Gain (ADG)
At all time points, bodyweights of pigs in groups T01, T02 and T03 were comparable. Up to 14 days after the 2nd vaccination, the mean bodyweight of T01 animals was slightly higher compared to T02 and T03.
ADG from castration to weaning, castration to slaughter and 1st vaccination to slaughter, respectively, was comparable in T01 and T02. However, ADG were significantly higher in T02 than in T01 from 2nd vaccination to slaughter (p=0.0002) and 2nd vaccination +14D to slaughter (p=0.0001).

Conclusions:
The study confirmed that Improvac induces significant titres of antibodies to GnRF, testosterone levels were similar to those in surgically castrated pigs. Androstenone and skatole concentrations in belly fat were lower for both surgically castrated and vaccinated groups compared with entire boars.

The CVMP noted that in most studies, the prevalence of animals with significant levels of skatole at slaughter was low in all treatment groups including entire boars. In addition, the group of boars was rather small, and only a small amounts of boars had skatole levels >200ng/g belly fat. This means that the conclusions to be made with regard to reduction of this important taint component are not as powerful as they would have been in a "worst case" scenario, where the problems with high skatole levels are more prevalent and more animals are showing the trait.

5) Study Title: Evaluation of the Efficacy and Economic Benefits of Improvac Vaccine in Controlling Boar Taint in Male Pigs under Commercial Field Conditions in Hungary
This was a GCP compliant study in which pigs were assigned to 3 treatment groups when 2 -13 days old. The treatment groups were as follows: T01: surgical castraed pigs; T02: Improvac vaccinated pigs. Vaccination was performed twice with a 4 – week interval. Pigs received 2ml Improvac per vaccination when aged 20–23 weeks. The second vaccination followed after 28 days at age 24–27 weeks, before the slaughter of animals; T03: included entire boars.
The product used was of minimum potency. All pigs were weighed at a few days of age and physically examined. Surgically castrated and vaccinated pigs were followed more closely around castration, weaning, and vaccination.

Follow-up
Efficacy was assessed by
-Measuring anti-GnRF antibodies, changes in testicular size and serum testosterone after vaccination
-Measuring androstenone, skatole and indole in belly fat at slaughter.
Impact of surgical castration vs. vaccination was followed by monitoring body weight, clinical abnormalities and carcass quality at slaughter.

Safety issues are discussed in part 3.
Any mortalities that occurred during the study period did not seem to be related to vaccination.

Results
A. Anti-GnRF antibodies
Treatment, time and the interaction of treatment and time all had a significant effect on the titre of antibodies against GnRF. Compared to T01 and T03, the proportion of pigs in T02 with no quantifiable antibody titres dropped significantly from 46.6 % to 2.7 % between 1st vaccination and 14D after the 2nd vaccination, and further to 0 at slaughter. This corresponded to a marked rise in mean quantifiable anti-GnRF titres among vaccinated pigs following the 2nd vaccination, with the highest anti-GnRF antibody levels measured 14 days later. At the time of slaughter, the quantifiable titres had declined again, but were still about six-fold the level of antibodies in castrated pigs and boars. In both T01 and T03, significant proportions of animals had detectable levels of antibodies, probably attributable to non-specific background reaction during the ELISA.

B. Testicular measurements
No significant difference was apparent between pigs from the two treatment groups at the time of 1st vaccination, or between left and right sides. At slaughter, it was evident that the increase of testicular size in entire boars was more pronounced than among vaccinated pigs. Mean testes length increased 41- 43 % in entire boars compared with 34 - 36 % in T02. Mean width increased 29 - 37 % in boars between 1st vaccination and slaughter, compared to 16 - 21 % in vaccinated pigs.

C. Testosterone levels LLOQ= 0.1 ng/ml
Most pigs from group T01 had serum testosterone levels BLOQ from 1st vaccination to slaughter.
Most samples from boars contained quantifiable levels of testosterone, and the geometric mean concentration rose from 1.66 ng/ml at 1st vaccination to 2.13 ng/ml at slaughter among entire boars with quantifiable testosterone concentrations.
At 1st vaccination, 2.7 % of vaccinated pigs had testosterone levels BLOQ, while the proportions at 2nd vaccination, 14 days after 2nd vaccination and at slaughter were 0, 65% and 56%, respectively. The

mean quantifiable levels fell from 0.94 ng/ml at 1st vaccination to 0.57 ng/ml at slaughter, the lowest level being 0.46 ng/ml 14 days after the 2nd vaccination.

D. Androstenone, skatole and indole
The LLOQ were 200 ng/g, 13.2 ng/g and 12.9 ng/g for androstenone, skatole and indole, respectively.
No T01 castrated pigs had androstenone levels above LLOQ in belly fat samples, while 13% T02 pigs and 76% entire boars had geometric mean levels of 337 ng/g and 696 ng/g, respectively.
All pigs regardless of treatment had detectable levels of skatole in the belly fat samples at slaughter. Quantifiable skatole levels were not significantly different in groups T01 and T02, about half the mean value measured in boar samples. All belly fat samples also contained quantifiable levels of indole, and no significant differences were evident between the geometric mean concentrations in the three groups.

Distribution of pigs by belly fat concentration of androstenone and skatole
Androstenone levels >1000 ng/g belly fat were only measured in entire boars and 44% of those also had high levels of skatole (>200 ng/g). All castrated pigs except two could be classified as having low levels of skatole (their androstenone levels were below LLOQ). This was one of the few studies in the dossier, where there are higher proportions of animals (24% of entire boars) with skatole levels in belly fat > 200ng/g.
Entire boars tended to have either higher androstenone or skatole concentrations, or both, than pigs from T01 and T02.

E. Carcass evaluation
The SEUROP Scoring system was used. Lean meat percentages were highest in boars, followed by Improvac vaccinated pigs, and then castrated pigs. The majority of T03 and T02 carcasses were graded next best, E. Most pigs from group T01 (65 %) were graded U after slaughter. No pigs were graded the lowest grading, P.
There were no statistically significant differences between entire boars and vaccinated pigs with regard to backfat thickness or muscle thickness. Mean muscle thickness was 4-6 % higher in T02 and T03 carcasses than in T01 pigs. Mean hot carcass weight in the vaccinated group was 3% lower than in T01 and 4% lower than in T03.

F. Bodyweight and Average Daily Gain (ADG)
Bodyweight (kg) ±SD, (no. of pigs)
Bodyweights of pigs in groups T01, T02 and T03 were comparable, with entire boars weighing slightly more than T01 and T02 pigs at all time points.

Least Square Means Average Daily Weight Gain (g) ±SD, (no. of pigs)
Least square means ADG from castration to weaning and from castration to slaughter was comparable in T01 and T02. Entire boars were found to have the highest average daily weight gains over the whole study period, which correlates with the highest mean bodyweights in this group.
The CVMP noted that is a known fact that entire boars have better feed conversion rate and daily growth compared with castrates. This is caused by the male hormonal activity. When Improvac vaccinated pigs decline in their male hormones during the last 4-6 weeks before slaughter they resemble the castrated pigs during this period both with respect to metabolism and behaviour. Therefore the choice of the Applicant for the window of 4-6 weeks between the last vaccination and slaughter was based on optimum slaughter carcass quality besides optimal reduction of boar taint rather than the actual duration of immunity of 10 weeks.
Conclusions:
Skatole levels seemed to be more variable among vaccinated pigs and entire boars, and there were also more pigs with medium androstenone levels than in the other field trials. However, they were still below the proposed threshold level. Considerable proportions of animals from all treatment groups had low-level skatole- and indole-positive fat samples, and indole concentrations were comparable in all three groups. In this study, the carcass quality results were similar for boars and vaccinated pigs. This is probably due to vaccination of the pigs at a comparatively relatively late point of time, which

means that they were more boar-like. Boars seem to perform best in regard to ADG and carcass quality.
Vaccinated males consistently had smaller testicular measurements compared with the entire boars and these measurements were in part influenced by the baseline measurement, as indicated by significant effect of first vaccination in the model.
Overall this was a well conducted pivotal study supporting the efficacy of the product.

Additional studies/data
In addition to the studies presented above, the Applicant provided summaries of two other studies carried out in Australia using batches manufactured by CSL. The CVMP considered that the batches used for these studies were experimental batches targeted to contain 400 µg antigen per dose but as they were not fully tested according to the standard batch release requirements it was therefore not possible to assess their relevance. They were not considered by the Applicant to be pivotal and were therefore not considered further. The Applicant has also referred to 4 published reports relating to studies on the efficacy of Improvac.

Finally, the Applicant identified sensory data comparing pork from vaccinated pigs to pork from surgical castrates as confirmation of the chemical data providing assurance of the comparable efficacy of vaccination to surgical castration for the control of boar taint. In this regard, the Applicant and third party researchers conducted 17 sensory studies (both expert trained panels and/or consumer panels) with the product throughout the world, including Europe, under different commercial, nutritional and climatic conditions and with different breeds of pigs. Summaries of these 17 studies were provided. Vaccinations were given at least 4 weeks apart with the second dose given 4-6 weeks prior to slaughter. Without exception, pork from vaccinated male pigs was found to be indistinguishable from pork from both surgical castrates and female pigs. In some cases, it was actually preferred. In addition to the chemical results for androstenone and skatole, these sensory data provide confirmation of the efficacy of Improvac as being comparable to the practice of surgical castration for the control of boar taint.

The CVMP concluded that the results were consistent with the pivotal efficacy studies carried out by the Applicant and as such can be considered supportive but cannot on their own substantiate a claim.

OVERALL CONCLUSION ON EFFICACY

In all studies, the vaccinated pigs were comparable to surgically castrated pigs in terms of boar taint compound concentrations at slaughter. The efficacy studies demonstrated that

a) while the first vaccination did not induce any specific anti-GnRF response, the second dose of Improvac resulted in significant levels of anti-GnRF antibodies, which peaked between one to two weeks post-injection and then declined, but still was significant relative to unvaccinated animals after four weeks. The proposed administration scheme includes two subcutaneous administrations with an interval of at least 4 weeks. The second injection should be given a minimum 4 weeks and a maximum of 6 weeks before slaughter, where androstenone and skatole levels are below the threshold proposed by the Applicant (Skatole 200 ng/g; Androstenone 500-1000 ng/g). Despite no definitive data on threshold levels can be adopted to categorise a carcass as free of boar taint acceptable among consumers in the EU, it could be accepted that the product controls levels of androstenone and skatole below those considered as clearly tainting. Immunological response is not dose-related.

b) In all pivotal field studies, the vaccinated pigs were comparable to surgically castrated pigs in terms of boar taint compound concentrations at slaughter.
Vaccination results in temporary suppression of testicular function, and during this period, concentrations of skatole and androstenone in belly fat at slaughter are below the proposed threshold levels, which reduces the risk of the occurrence of boar taint.

In conclusion, the Applicant provided adequate evidence for the main efficacy claims in the laboratory and field trials submitted. From the data presented the CVMP concluded that there should be little difference in the numbers of "non-responding" surgically castrated or Improvac vaccinated pigs.

V. BENEFIT-RISK BALANCE

The data provided within Part II, III and IV were considered satisfactory and current guidelines were taken into account. Formulation development was well described and the product composition justified. The product used in pivotal clinical efficacy and safety studies was shown to be equivalent to the formulation proposed for marketing. Details of the manufacturing process and process validation were provided which show that product of the desired quality can be consistently produced by the process as described. The release specification is well designed to control the quality of the product, and the stability studies support the proposed shelf-life. The starting materials used in the production of the final product have been declared in compliance with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Council Directive 2001/82/EC as amended. A User Safety assessment was provided and the user risk management procedures detailed in section 4.5 of the SPC are appropriate. Safety studies were conducted to sufficiently support the safety profile of the product. The efficacy of the product was supported in a pivotal laboratory study and in five field studies performed in Europe.

The main benefits of the product can be summarised as follows:
i) A significant improvement in the overall health and welfare of male pigs raised for human consumption
Surgical castration is a procedure that is being used on approximately 100 million male pigs in the EU every year to control boar taint, predominantly without any form of anaesthesia or analgesia. This procedure is universally accepted as being painful and potentially having a highly adverse effect on the health and well-being of these sentient animals. On both welfare and ethical grounds, other options are being urgently sought in the EU and other European countries to significantly curtail, or even eliminate, this undesirable practice. As a method of boar taint control, vaccination with Improvac offers a viable well-proven alternative to surgical castration; one that has already been adopted in several major pig producing countries in the world and, contrary to the practice of surgical castration, involves minimal pain and risk to the vaccinated pig. Vaccination with Improvac will also prevent those pre-wean infections (e.g., *Streptococcus suis*, *Haemophilus parasuis*) and mortalities (comprising approximately 1% of male pig population or approximately 1 million pigs/year in the EU) that occur as a consequence of surgical castration.
ii) Potential reduction in the amount of antibiotics used overall in pig production
The use of vaccination will prevent those pre-wean infections (e.g., *Streptococcus suis*, *Haemophilus parasuis*) which occur as a consequence of surgical castration and should thus lead to an overall reduction in the amount of antibiotics used in pig production, although this has not been proven.
iii) Potential decrease in incidence of highly tainted pigs (due to cryptorchidism) entering the human food supply
There is a high potential that the incidence of highly tainted pigs entering the human food supply could actually be reduced with the use of vaccination in place of surgical castration, assuming similar compliance rates for vaccination and surgical castration, due to the fact that Improvac will be able to control boar taint in cryptorchid males (approximately 500,000 produced every year in the EU).

iv) Elimination of the need for farm workers to physically castrate pigs with sharp blades
To the user (veterinarian, pig farmer) the alternative of vaccination eliminates the unpleasant task of surgical castration and the need for farm workers to use razor sharp blades to castrate piglets under relatively unsanitary conditions, thereby reducing the risk of injury and infection.

Risks include the vaccination procedure of two 2 ml subcutaneous injections immediately behind the ear. Pigs showed little reaction to the act of injection and injection site reactions and other post-vaccination effects, as described in the SPC, are relatively mild. In contrast to surgical castration,

this procedure involves minimal pain and negligible risk to the pig. The main risks to the user are associated with accidental self-injection. However the risks can be minimised as the product is a Prescription Only Medicine. Supply of the product will be under veterinary control and measures for safe handling have been introduced. The Applicant has ensured, and will continue to ensure, that safety vaccinators are readily available to each market prior to the introduction of Improvac. As part of assuring the safe and effective use of the product, the Applicant will also emphasise the need for effective training for all users of the product. The Applicant will supply training materials and in-country technical staff will either train farm staff directly or train the relevant veterinarians to train these users.

No major risks could be identified by the use of the product according to the SPC.

On the basis of the above, the overall benefit risk analysis was deemed positive with a sufficiently clear and complete SPC and product literature.

Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of Improvac were considered to be in accordance with the requirements of Council Directive 2001/82/EC, as amended.