SCIENTIFIC DISCUSSION

1. SUMMARY OF THE DOSSIER.

Leucofeligen FeLV/RCP is a biotechnology derived immunological veterinary medicinal product intended for the vaccination of cats against Feline Calicivirus (FCV), Feline Viral Rhinotracheitis virus (FVR), Feline Panleucopenia virus (FPV) and Feline Leukemia virus (FeLV). It is intended for the active immunisation of cats from eight weeks of age against:

- feline calicivirosis to reduce clinical signs.
- feline viral rhinotracheitis to reduce clinical signs and viral excretion.
- feline panleucopenia to prevent leucopenia and to reduce clinical signs.
- feline leukaemia to prevent persistent viraemia and clinical signs of the related disease.

Leucofeligen FeLV/RCP contains three live attenuated viral strains (FCV, FVR and FPV) and a purified p45 recombinant protein derived from the gp70 surface glycoprotein of the FeLV. The final association includes aluminium hydroxide and highly purified extract of *Quillaja saponaria*. The vaccine consists of a lyophilisate: Feligen RCP and solvent for suspension for injection, Leucogen. Leucogen liquid fraction is a highly purified vaccine against feline leukaemia produced by genetic engineering. A sequence of the envelope protein of FeLV is introduced via a plasmid into an appropriate *Escherichia coli*. FeLV envelope p45 protein is initially expressed in large quantities in inclusion bodies. These bodies are extracted and thoroughly purified. The final preparation includes the addition of aluminium hydroxide and highly purified extract of *Quillaja saponaria*. It is presented in vials containing one dose of 1ml as liquid preparation. Since the vaccine itself is highly purified and does not contain retraceable DNA, there are no concerns regarding potential recombination or reassortment.

2. QUALITY ASSESSMENT

Composition

The liquid fraction Leucogen is an adjuvanted liquid vaccine containing a purified recombinant p45 protein derived from the gp70 surface glycoprotein of the FeLV subgroup A, expressed in *Escherichia coli*. Each dose of 1 ml contains at least 102 µg of purified p45 recombinant product. The antigenic suspension is adjuvanted with an aluminium hydroxide gel and a purified extract of *Quillaja saponaria*. The lyophilisate contains live attenuated feline calicivirus (strain F9) $10^{4.6}$-$10^{6.1}$ $CCID_{50,}$ (Cell Culture Infectious Dose 50 %), live attenuated feline viral rhinotracheitis virus (strain F2) $10^{5.0}$-$10^{6.6}$ $CCID_{50}$ and live attenuated feline panleucopenia virus (strain LR 72) $10^{3.7}$-$10^{4.5}$ $CCID_{50}$.

Container

The vaccine is filled in 3 ml insulin type vials Type I. The vials are closed with butyl elastomer stoppers and sealed with perforated aluminium capsules. This method of closure and opening is in conformity with the current edition of the European Pharmacopoeia.

The vials and stoppers are sterilised. Filling and capping were described and the certificates of the vials and stoppers were presented. The method of sterilisation described was satisfactory.

Development Pharmaceutics

The choice of the F9 feline calicivirus strain, the LR72 feline parvovirus strain and the F2 feline rhinotracheitis virus strain (Herpesvirus) were described. Concerning choice of the FeLV antigen, the feline leukaemia virus (FeLV) antigen is a naturally occurring pathogenic retrovirus contagious for pet cats. The FeLV genome contains amongst the *gag* and *pol* genes the *env* gene that encodes the envelope protein composed of a glycoprotein of 70,000 dalton (gp 70) and a 15,000 dalton protein

(p15E). Gp 70 is essential for binding of the virus to the cellular receptors for FeLV. Due to the importance of the gp 70 in protection, gp 70-vaccines were developed. These predecessor vaccines were either not associated with the production of virus neutralising antibodies, or did not protect against FeLV challenge. In contrast, promising results were anticipated using a recombinant antigen that derived from the entire gp 70 gene supplemented by an effective adjuvant to increase the immune response, especially against FeLV-subgroup A. Consequently, a vaccine was developed containing purified recombinant p45 protein that was derived from the gp70 surface glycoprotein of the FeLV subgroup A and is expressed in *Escherichia coli,* supplemented by effective adjuvants to increase the immune response. The p45 structure is adsorbed on to aluminium hydroxide gel and a highly purified extract of *Quillaja saponaria*, both acting as adjuvant.

The process implementation was described.

Concerning choice of the pharmaceutical form and formulation, the vaccine is composed of two flasks to be associated extemporaneously:

The freeze-dried flask comprises three components: calicivirus, rhinotracheitis virus and panleucopenia virus, some of them are very fragile. The cryodessication ensures a better conservation and allows preserving them in an easily useable form. The composition of the excipient is described and comprises:
- a sugar that fixes the residual humidity
- a viral protective agent
- a texturing agent.

The liquid flask comprises the p45 recombinant molecule as an active substance.

The antigenic epitope responsible for anchoring to cell receptors and immune protection is localised on the protein component of glycopotein gp70; it is conserved on the p45 molecule produced by *E. coli.* The p45 structure is therefore adsorbed onto an adjuvant, consisting of aluminium hydroxide gel and a highly purified extract of *Quillaja saponaria* called QA21. Both ingredients allow for a correct presentation of the p45 in space and improve the immunological response.

The excipient of the liquid fraction of the vaccine is a buffered saline solution. This excipient allows for the adjustment of the proteic concentration required for the final product.

Clinical trials formulation

The composition of the batches used in the clinical trials was described and they were produced according to the manufacturing methods described in the dossier.

Method of manufacture

The applicant confirmed that the active substances are manufactured according to GMP.

Flow charts for manufacture of the **freeze-dried fraction** were presented. Data were presented on the composition of the bulk vaccine.

Filling is performed in a sterile area. After filling, the vials are pre-stopped with rubber stoppers previously sterilized. The vials are inserted into a previously decontaminated freeze-drier. Critical steps of the freeze-drying process were described. Sealing and coding is performed in a negative pressured room. They are sealed with an aluminium capsule and then immediately coded. The sealed vials are stored in a cold room.

Flow charts for manufacture of the **liquid fraction** were also presented. The biosynthesis in *Escherichia coli (E. coli)*, purification and control of the p45 FeLV envelope antigen are described. A stability study performed reveals a shelf life of 48 months.

A detailed description of these production processes was provided including information on the relevant formulas. Preparation and control of the recombinant *E. coli*, and of the p45 FeLV envelope antigen including the genetic engineering steps were described.

Corresponding flow charts of the latter production steps were provided with regard to the formulation, the blending and filling of the finished product including bulk preparation, preparation of vials and stoppers, and filling as well as packaging.

Relevant details were provided on the exact composition of the bulk vaccine.

A detailed description was provided for the preparation of the vials and stoppers as well as of the filling, crimping, and coding. The method of preparation at Virbac Carros, France was clearly described. Further information on controls and results were presented. Relevant in-process stability data were provided along with information on the storage period and relating stability data.

Validation studies

The validation of the critical steps of the process was carried out.
Details were provided of the studies to demonstrate the batch to batch consistency of the manufacturing process. Results of batches were presented.
Reproducibility of the process was confirmed and the production process was considered to be consistent.

Information was provided for the validation of the sterilisation of the vials as well as for the validation of the filling process including tabular overviews. The results obtained were satisfactory.

CONTROL OF STARTING MATERIALS

Concerning starting materials listed in a Pharmacopoeia, certificates of analysis were provided for the components of the freeze-dried fraction and liquid fraction. The certificates of compliance provided were satisfactory.

Starting materials of biological origin not listed in a Pharmacopoeia included cells used for production of Feline Rhinotracheitis virus, Calicivirus and Panleucopenia virus. Relevant information was provided on the cell line, the current Master Cell Seed and the Working Cell Seed.

The preparation of the Master Cell Seed was described in detail and a detailed flow chart of the procedure was provided. Preparation of the Working Cell Seed was also described and a flow chart provided. Details of controls and tests carried out on Master Seed and Working Seed were also described, including: general microscopy, absence of bacteria and fungi, absence of mycoplasma, absence of extraneous agents, identification of the species, karyotype and tumorogenicity

Certificates of analysis for Master and Working Cell Seed were presented and the results of the tests performed were acceptable. The Applicant confirmed that the test for sterility is performed according to Ph.Eur. 2.6.1 and the test for mycosplasma according to Ph.Eur. 2.6.7.

Details were provided on the feline calicivirus strain used for production of the vaccine against feline Calicivirosis. Preparation of the Working Seed was described as well as the amplification steps performed. Controls and tests carried out on the Working Seed were described: absence of bacteria and fungi, absence of mycoplasma, absence of extraneous agents and identification of the species.

A certificate of analysis for feline calicivirus Working Seed was presented. Detailed information about methods and material used for detection of extraneous agents was included. Tests performed on the MSV were described. A certificate of analysis for feline Calicivirus Master Seed was presented.

Production of the Calicivirus suspension was described and detailed flow charts were presented.

The following *in-process* control tests were conducted: routine control of the production cells, sterility, mycoplasma, extraneous virus and titre. Certificates of analysis for recent batches were provided and the test results were acceptable.

A description was provided of how stability of the viral suspension was tested. The claimed shelf life for inocula and harvests was considered acceptable based on the test results provided.

Details were provided on the feline rhinotracheitis virus strain used for production of the vaccine against feline rhinotracheitis, as well as how the Working Seed is prepared and the steps of amplification during production. A detailed flow chart was provided as well as details of the controls and tests carried out on the Working Seed: absence of bacteria and fungi, absence of mycoplasma, absence of extraneous agents and identification of the species.

A certificate of analysis for Feline Rhinotracheitis virus Working Seed was presented. Detailed information about the methods and materials used for detection of extraneous agents was also provided. Tests performed on the MSV were described.

Production of the Rhinotracheitis virus suspension was also described in detail. Detailed flow charts were presented. The following *in-process* control tests were conducted: routine control of the production cells, sterility, mycoplasma, extraneous virus and titre of final suspension. Certificates of analysis for recent batches were provided. The test results were acceptable. The claimed shelf life for harvests was acceptable based on the test results provided.

Details were provided on the feline panleucopenia virus strain used for the production of the vaccine against feline panleucopenia. The Working Seed preparation and amplification steps were described.

The following controls and tests were carried out on the Working Seed: absence of bacteria and fungi, absence of mycoplasma, absence of extraneous agents and identification of the species.

The Certificate of analysis for Feline panleucopenia virus Working Seed was presented. Detailed information about the methods and material used for detection of extraneous agents was provided. Tests performed on the MSV were described and a certificate of analysis for feline panleucopenia Master Seed is presented.

Production of the Panleucopenia virus suspension was described in detail. Detailed flow charts were presented. The following *in-process* control tests were conducted: routine control of the production cells, sterility, mycoplasma, extraneous virus and titre.

Certificates of analysis for recent batches were provided. The test results were acceptable. Concerning stability of the viral suspension and the claimed shelf lives for inoculum and harvest, the test results and the claimed shelf lives were acceptable.

Concerning the FeLV-component, details were provided on the *E. Coli* strain used as the host strain for expression of the FeLV envelope recombinant antigen.

The construction of the plasmid expression vector was described, along with the culture medium and the strain used for the construction of a seed lot system. An overview was provided on the Master and Working Cell Banks as well as storage conditions.

The following controls are carried out on master and working seed lots:

- Cell viability
- Auxotrophic markers
- Contamination with other micro organisms
- Restriction mapping of isolated plasmid
- Western blot analysis of induced proteins
- DNA sequencing.

Detailed descriptions were provided on the controls carried out. Validation of Master and Working seeds following fermentation was also described. Concerning the genetic engineering aspects, the gene of interest is the DNA which encodes the FeLV envelope antigen. Sourcing and preparation of the genomic DNA were described. The preparation of the production strain and steps involved were described and a flow chart was provided detailing each step.

The process for cloning DNA which encodes the FeLV envelope antigen was also described. Preparation and construction of the expression vector were detailed, along with cloning for expression, introduction into the starting strain, description of the production cell line, expression, genetic, constructional and segregational stability and stability up to and beyond the maximum passage level. Production and control of the p45 FeLV envelope antigen were described along with the control tests performed. Detailed information was provided with regard to the analytical reagents used and relevant certificates of analysis were included.

A description was provided of in-process controls and validation, with a description and control of the p45 FeLV envelope antigen production process, amplification/ culturing, purification, preparation of the purified p45 envelope antigen, as well as evidence of batch-to-batch-consistency.

Control tests during production were also described and relevant validation data were included.

Starting material included in the composition of the media

A comprehensive list of the starting materials included in the composition of the media was provided which specified the internal codification of each product. Each starting material was described and specification sheets were supplied. Certificates of analysis and supplier's documentation were provided.

Satisfactory specifications and certificates of analysis were also provided for the starting materials of non-biological origin:

In-house preparation of media was described and detailed information was provided on the composition, the method of preparation, storage conditions and storage life as well as controls and tests carried out.

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

Assessment of starting materials has been conducted in compliance with Commission Directive 1999/104/EC and in accordance with Guideline EMEA/CVMP/410/01 Rev. 2 OCT 2003 (2004/C 24/03): "Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products", and also taking into account Eur. Ph. monograph 01/2005:1483: "Products with risk of transmitting agents of animal spongiform encephalopathy" and related chapter 01/2005:50208 and position paper EMEA/CVMP/019/01: "Assessment of the risk of transmission of animal spongiform encephalopathy agents by master seed materials used in the production of veterinary vaccines"

For the starting materials of animal origin or including an animal origin component used for vaccine production satisfactory TSE Certificates of Suitability according to the Ph.Eur. Monograph were provided.

Materials were chosen to minimise the risk of transmission of TSE agents and the starting materials of animal origin used in the production of the final product comply with the current guidelines and regulatory texts related to the TSE Guideline EMEA/CVMP/410/01 Rev. 2.

CONTROL TESTS DURING PRODUCTION

For the freeze-dried fraction a flow chart of the bulk preparation was presented, along with details of the controls performed during production: calculation of the needs – weighing of the constituents, starting materials, control of the tank, preparation of the vials, preparation of vacuum stoppers, filling, freeze-drying and packaging. The test results of various batches were provided and an example batch protocol for the in-process controls was provided. For the liquid fraction control tests for the various manufacturing steps were described.

Each test was described and a limit of acceptance was provided, if applicable. If relevant, the corresponding SOP was mentioned. Batch to batch consistency related to these steps was demonstrated and results of a number of batches were presented.

CONTROL TESTS ON THE FINISHED PRODUCT

Control tests carried out on the freeze-dried and liquid fraction were described, including physico-chemical characters (aspect, volume, pH and solubility for the freeze-dried fraction), a vacuum test to demonstrate that the freeze-dried vaccines present a vacuum at the end of the manufacturing process, residual humidity, identification for calicivirus, rhinotracheitis virus and panleucopenia virus, sterility, mycoplasma, extraneous virus, titre and safety. Specifications and batch results were provided.

Timing and frequency of testing were indicated. As well as a description of the tests related SOPs and associated validations were provided.

STABILITY

Stability of the bulk antigen

The stability of the FCV, FVR and FPV antigens was set as 48 months at ≤ -35° C.
The stability of the FeLV p45 antigen was set for up to 48 months.
All relevant details were provided.

Stability of the finished product

Freeze-dried fraction:

A shelf life of 24 months at 2° C – 8° C was claimed and confirmed by testing stability over a period of 27 months. Tests carried out included aspect, pH, residual humidity, and virus titre, sterility, mycoplasma and safety.

Liquid fraction:

The stability of the finished product was documented demonstrating stability of batches of Leucogen stored for 27 months at + 4° C.

Tests performed included: physico-chemical parameters (aspect, volume, pH), identity/purity, sterility, protein titration, potency, and safety. The relevant SOPs were mentioned and brief test descriptions were provided including limits of acceptance.

The data provided show the stability of the product for the period claimed. A shelf life of 24 months was fully justified. Virbac S.A. committed to conduct a follow-up stability study with regard to the adjuvant content as a follow-up measure.

Stability of the reconstituted product

To demonstrate the stability of the reconstituted product at room temperature a study was carried out. No significant loss in titres was observed. As the SPC requests to use the vaccine immediately after reconstitution, this study was accepted for demonstration of the stability of the reconstituted vaccine.

DATA RELATED TO THE ENVIRONMENTAL RISK ASSESSMENT FOR PRODUCTS CONTAINING OR CONSISTING OF GENETICALLY MODIFIED ORGANISMS

As this vaccine does not contain a GMO capable to replicate in the environment but a properly defined not viable recombinant protein, this part is not applicable for the evaluation of the product in question.

OVERALL CONCLUSION ON QUALITY

Freeze-dried fraction:

The analytical dossier was considered acceptable. Based on the production process described and the starting materials used including all relevant tests, it was shown that a vaccine of consistent quality can be produced. Controls during manufacture and tests on the finished product should guarantee the compliance with the quality parameters mentioned. All test instructions are provided as SOPs and validation studies have been performed. The claimed shelf-life of 24 months storage at 2–8 °C is acceptable.

Liquid fraction:
Leucogen is a genetically engineered vaccine containing as active ingredient the highly purified protein p45 envelope antigen which is expressed by an appropriate *E. coli*. Efficient adjuvants are added (aluminium hydroxide gel and Purified extract of *Quillaja saponaria*).

All relevant directives, guidelines and monographs as well as related EMEA position papers and VICH guidelines were taken into account. The description of the plasmid construction and its insertion into the *E. coli* host strain is comprehensible. Identity, purity and stability are well documented. Results of *in-process* controls demonstrate consistency of the production process and the final product testing results indicate consistency. The methods used for final batch testing are properly validated.

All starting materials used are well documented and tested before their use. Any potential TSE risk is discussed and considered to be nil. The stability of the product is demonstrated for several production steps and for the final product. 24 months are fully justified. The production of a consistent vaccine of high quality is ensured under the conditions documented.

3. SAFETY ASSESSMENT

INTRODUCTION

Leucofeligen FeLV/RCP is a combined vaccine containing the freeze-dried Feligen CRP vaccine with the liquid adjuvanted Leucogen vaccine. Feligen CRP is composed of three

live attenuated antigens: Feline Calicivirus (FCV, strain F9, $10^{4.6}$ to $10^{6.1}$ $CCID_{50}$), Feline Rhinotracheitis virus (FVR, strain F2, $10^{5.0}$ to $10^{6.6}$ $CCID_{50}$) and Feline Panleucopenia virus (FPV, strain LR72, $10^{3.7}$ to $10^{4.5}$ $CCID_{50}$). This freeze-dried fraction is reconstituted before use with the Leucogen monovalent adjuvanted liquid component. The Leucogen fraction contains the purified recombinant p45 protein derived from the gp70 surface glycoprotein of the FeLV subgroup A, expressed in *Escherichia coli*. The Leucogen fraction contains not less than 102 µg of purified p45 recombinant protein.

Leucofeligen FeLV/RCP is intended for the immunisation of kittens against Feline Calicivirosis, Feline Rhinotracheitis, Feline Panleucopenia, and Feline Leukaemia. The regimen of vaccination recommends two subcutaneous injections of one dose of Leucofeligen FeLV/RCP at a three to four week interval in kittens from eight weeks of age (basic vaccination scheme). An annual booster immunisation with one dose of Leucofeligen FeLV/RCP is recommended (re-vaccination scheme).

Feligen CRP and Leucogen were first commercialised separately in the 1980s (Feligen CRP in 1981 in Germany, and Leucogen in 1988 in France). Since then, both vaccines have been granted marketing authorisations in other European countries. Laboratory and field trials were performed for the renewal of these vaccines in the 1980s and 1990s to demonstrate their safety and efficacy in accordance with the requirements in force at that time. Additional studies have been undertaken more recently using the combination of these two vaccines in order to support the safety of Leucofeligen FeLV/RCP according to the current legislation.

GENERAL REQUIREMENTS

As Leucofeligen FeLV/RCP is a vaccine intended for cats only, the safety tests have been carried out in the feline species (SPF cats). Leucofeligen FeLV/RCP is intended for kittens from eight weeks of age. For animal welfare reasons, the animals used in most of the laboratory studies were not vaccinated at the youngest recommended age but slightly older. Nevertheless, the minimum age of 8 weeks recommended for this vaccination is supported by data from the field safety study, in which kittens were exactly 56 to 63 days days old. The data presented were sufficient to claim a minimum age for primary vaccination “from eight weeks of age”.

The administration of Leucofeligen FeLV/RCP is recommended via the subcutaneous route. This route of administration was therefore used in all the laboratory and field safety studies. One dose of Leucofeligen FeLV/RCP is given twice at a three to four week interval to kittens from eight weeks of age (basic vaccination scheme). An annual booster immunisation using one dose of Leucofeligen FeLV/RCP is recommended (revaccination scheme). The vaccinations performed during the laboratory and field studies thus followed this vaccination scheme.

All viral suspensions and doses of Leucofeligen FeLV/RCP used for the safety studies originated from batches produced in accordance with the manufacturing process described by the manufacturer. The batch protocols or certificates of analysis for all the products used in these safety studies are provided in the final report of each trial.

Safety of the administration of one dose and the repeated administration of one dose.

The safety of the single and the repeated administration of one dose of Leucofeligen FeLV/RCP were assessed in the same study. Thereby, cats received one dose of Leucofeligen FeLV/RCP (i.e. one dose of the Feligen CRP fraction reconstituted with one dose of the Leucogen fraction) subcutaneously. Two additional single dose administrations of this vaccine were performed at a three-week interval.

For each cat, daily general and local clinical examinations were carried out. The rectal temperature was measured the day before the first injection, after each injection and during each clinical examination. The cats were weighed once a week. Blood sampling was performed prior to vaccination to confirm that cats were seronegative towards the four vaccine valences.

The serological analyses confirmed that all the kittens were free from antibodies against Feline Calicivirus, Feline Rhinotracheitis virus, Feline Panleucopenia virus and Feline Leukaemia virus before the first vaccination. After the first vaccine administration, all cats remained in perfect health and showed a regular bodyweight increase during the follow-up. No general reaction or significant variation of the rectal temperature was observed.

At the injection site, a moderate oedema occurred in many cases, followed by a swelling which could turn into a nodule. All these reactions were $\leq$ 2cm and disappeared spontaneously within 3 to 4 weeks at the latest. Local reactions observed after the second and third injections were weaker than those seen after the first vaccination. This study thus demonstrates the safety of the administration of one dose as well as of the repeated administration of one dose of Leucofeligen RCP/FeLV.

Safety of the administration of an overdose.

In this study, cats received by the subcutaneous route one injection of an overdose of the vaccine corresponding to ten doses of the Feligen CRP fraction reconstituted with two doses of Leucogen and two doses of diluent.

For each kitten, general and local clinical examinations were carried out daily during 14 days after the vaccination, or until reaction(s) had disappeared. The rectal temperature was measured the day before the injection, after the injection and during each clinical examination. The cats were weighed once a week until the end of the follow-up (or until disappearance of the local signs). Blood sampling was performed prior to vaccination to check that cats were seronegative towards the four vaccine valences.

The serological analyses confirmed that all the kittens were free from antibodies against Feline Calicivirus, Feline Rhinotracheitis virus, Feline Panleucopenia virus, and Feline Leukaemia virus before the first vaccination

This overdose administration did not induce systemic reactions except for transient hyperthermia for two days at the most. No local reactions other than those observed after injection of a single dose were noticed but they lasted longer (five to six weeks at the most). This study thus demonstrates the safety of an overdose administration of Leucofeligen FeLV/RCP (i.e. ten doses of Feligen CRP reconstituted with two doses of Leucogen and two doses of diluent).

Examination of reproductive performance

As Leucofeligen FeLV/RCP is not intended for use in pregnant animals, no study on reproductive performance has been conducted. A specific contra-indication to that effect is included within the Summary of Product Characteristics (SPC).

Examination of immunological functions

Leucofeligen FeLV/RCP contains three live attenuated viral strains (FCV, FVR and FPV) and a purified p45 recombinant protein derived from the gp70 surface glycoprotein of the FeLV. As Panleucopenia and Leukaemia wild viruses are known to induce immunosuppression, the effect of the corresponding vaccinal components on immunological functions have to be assessed (i.e. the panleucopenia vaccinal strain and the p45 recombinant protein).

FPV component:

Feline Parvovirus has active replicative properties in dividing cells including the lymphoid cells that could therefore induce disorders upon the immunological functions. The effect of the Panleucopenia

vaccinal strain on immunological functions was assessed in a number of studies through the follow-up of leucocyte numbers and/or thymus histological analysis after an overdose administration of the FPV vaccinal strain or vaccines containing this valence at ten times the maximum titre.
These studies demonstrate that an overdose of the FPV component of the vaccine has no negative impact on the immunological functions since cats remained healthy and no leucopenia nor thymus lesion were seen. The requirements of the Eur. Ph. Monograph are fulfilled satisfactorily.

FeLV component:

The vaccine contains a purified p45 recombinant protein which is derived from the gp70 surface glycoprotein of the FeLV. As the Feline Leukaemia wild virus is known to induce immunosuppression, the effect of the recombinant protein of the vaccine has been assessed.

It was shown that the peptide responsible for this immunosuppression is not contained in Leucogen.

Reference articles were presented.

Special requirements for live vaccines

Spread of the vaccine strain

Reversion to virulence studies presented provide information on the potential of these strains to spread by searching for them in the appropriate target organs after an overdose administration (i.e. at least 10 times the maximum virus titres contained in a Leucofeligen FeLV/RCP dose):
- Calicivirus was isolated in the respiratory system of one out of four cats (nasal mucus, tonsils and trachea) five days after vaccination,
- Rhinotracheitis virus was not isolated in the respiratory system of the four vaccinated cats (nasal mucus, tonsils, bronchial and pharyngeal lymph nodes, and trachea) four days after vaccination,
- Panleucopenia virus was isolated in the faeces of the two vaccinated cats three to ten days after vaccination.

These studies showed the absence of dissemination of the Rhinotracheitis vaccine strain through the organism of the vaccinated cat and thus its inability to spread to unvaccinated animals. Even if a very low spread of Feline Calicivirus or Feline Panleucopenia virus could occur, infection of other animals with these vaccinal strains could be considered as unable to induce high viral replication and disease. An appropriate warning concerning the possible excretion of FCV and FPV is included in the SPC.

Dissemination in the vaccinated animal

Specific studies have been undertaken to examine the possible reversion to virulence of the three live attenuated strains of Leucofeligen FeLV/RCP vaccine (FCV, FVR, FPV) taking into account the tropism of each strain. These studies provided detailed information on the vaccine strains dissemination in the body, with particular attention to the predilection sites for replication of these viruses. They showed the very restricted replication of the Calicivirus vaccinal strain and the absence of dissemination of the Rhinotracheitis virus vaccinal strain in the target respiratory organs. Although the Panleucopenia vaccinal strain was detected in the faeces, this strain does not show any typical signs of virulence of the parvoviruses. Finally, as the vaccine is not intended for food-producing animals, specific studies are not required.

Reversion to virulence of attenuated vaccines

Feline Calicivirus

A study was performed on the irreversibility of the attenuation of the F9 Feline Calicivirus vaccinal strain. This study demonstrated the absence of reversion to virulence of the Feline Calicivirus strain of Leucofeligen FeLV/RCP because no evidence of an increase of virulence (very restricted viral

replication and no clinical sign) was seen after a ten dose administration of the FCV vaccinal strain at the maximum titre and serial passages through cat.

Feline Rhinotracheitis virus

A study was performed on the irreversibility of the attenuation of the F2 Feline Rhinotracheitis vaccinal strain. The results showed that the Feline Rhinotracheitis virus vaccine strain of Leucofeligen FeLV/RCP did not revert to virulence because no evidence of viral excretion was seen after a ten-dose administration of the FVR vaccinal strain at the maximum titre.

Feline Panleucopenia virus:

A study was performed on the irreversibility of the attenuation of the Feline Panleucopenia vaccinal strain. This study demonstrated that the Feline Panleucopenia vaccine strain did not revert to virulence compared to the original Feline Panleucopenia virus after a ten dose administration and serial backpassages through cats since:
(1) No systemic abnormal reactions attributable to the vaccine strain were observed;
(2) No significant leucopenia occurred;
(3) No Parvovirus-induced thymus lesion was seen;
(4) The strain remained genetically stable throughout passages.

Biological properties of the vaccine strain

The different studies performed to examine the possible reversion to virulence of the three live vaccine strains of Leucofeligen FeLV/RCP vaccine (FCV, FVR, FPV) showed no modification of their tropism or increase of virulence of these attenuated viruses after serial passages in cats. Therefore, no specific studies were conducted to determine the intrinsic biological properties of the vaccine strains.

Recombination or genomic reassortment of strains

In the course of a virus replication/multiplication, natural forces of evolution are inevitably present and errors will occur, generating various types of "mutant" progenies. Feline Herpesvirus, Calicivirus and Panleucopenia virus strains are able to replicate in vaccinated cats. Even if this replication is very low, the risk of mutation or recombination events is not negligible. If such a rare event occurs, the worst scenario is the fortuitous co-infection with a virulent strain the worst consequence of which would be the reversion to wild-type virulence, causing a usual disease for the co-infected cat.

Study of residues

As Leucofeligen FeLV/RCP vaccine is an immunological product and is not intended for immunisation of food producing animals, no investigation regarding residues was undertaken.

Interactions

No information is available on the safety and efficacy from the concurrent use of this vaccine with any other. Therefore, no corresponding studies were undertaken. The use of immunosuppressive drugs (e.g. corticosteroids) close to vaccination with Leucofeligen FeLV/RCP should be avoided since it may interfere with the induction of immunity.

FIELD STUDIES

A field safety study of Feligen CRP / Leucogen vaccine in 8/9 week old kittens was performed.

Kittens at the youngest recommended age were selected from and each cat received the basic vaccination scheme by the subcutaneous route:
-One dose of Leucofeligen FeLV/RCP vaccine (i.e. one dose of Feligen CRP reconstituted with one dose of Leucogen at 8 to 9 weeks of age, first vaccination on D0),
-One dose of Leucofeligen FeLV/RCP vaccine, at 11 to 12 weeks of age, on a different injection site (second vaccination, on D21).

Each animal was followed for 35 days. During each vaccine period (first vaccine period from D0 to D21 and second vaccine period from D21 to D35), four or eight veterinary examinations were performed by the investigator, including rectal temperature and body weight measurements. Between each visit, owners carried out a daily clinical observation. The cats were thus examined or observed daily for signs of abnormal local and systemic reactions for each vaccine period. Blood was collected on the day of the first injection to determine the serological status of the kittens prior to vaccination.

Before vaccination (on D0), all kittens were seronegative to FeLV. 19, 48 and 22 % of them were seropositive to Feline Rhinotracheitis virus, Calicivirus and Feline Panleucopenia virus respectively.

This study demonstrates the safety of the vaccination with Leucofeligen FeLV/RCP under field conditions, because:
(1) the cats remained in good health after each injection;
(2) only slight and transient systemic reactions, commonly reported with the parenteral vaccination, were observed (mainly weakness and diarrhoea);
(3) no abnormal local reaction was observed at the injection site compared to the results of laboratory studies (transient and moderate oedema, swelling and/or nodule). The pain at palpation noted in field animals did not occur in the laboratory animals, and was therefore mentioned in the SPC.

Since the cats were aged from eight weeks on the day of the first injection, this data also supports the minimum age recommended for this vaccination.

PSUR:
The pharmacovigilance reports of Feligen CRP and Leucogen were presented.

VIRBAC Laboratories have been granted Marketing Authorisations for Feligen CRP and Leucogen in several Members States of the European Community since 1981 and 1988 respectively. Pharmacovigilance surveys have been conducted in these European countries for many years. The incidence of all reported suspected adverse drug reactions relating to Feligen CRP and Leucogen administration is very low. Considering these field data for the separate use of Feligen CRP and Leucogen vaccines and the studies performed with the combined Leucofeligen FeLV/RCP vaccine, the reactions expected with this vaccine in the field should be similar to those reported with separate vaccines, and thus with a very low incidence.

ENVIRONMENTAL RISK ASSESSMENT

The Leucofeligen FeLV/RCP vaccine is composed of two vials: the freeze-dried vial of Feligen CRP contains three attenuated live virus strains (FCV, FVR, FPV) which are of feline origin. Their attenuation was performed by successive passages on cat cells.
Feline Panleucopenia virus strain is excreted in faeces of vaccinated cats and it is considered to be a resistant virus. It can subsist, under certain conditions, from a few days to a few months in the external environment. Therefore, the risk and consequences of the diffusion of the strain to non-vaccinated animals have to be taken into account.

Feline Calicivirus strain is also considered to be a resistant virus which can subsist in the external environment under certain conditions, for a few days. Therefore, the risk and consequences of the diffusion of the strain to non-vaccinated animals have to be taken into account.
The Feline Rhinotracheitis virus strain is considered as a non-resistant virus. Therefore, the risk of diffusion of the strain to non-vaccinated animals is very low.

A phase I environmental risk assessment was provided. On the basis of this, a phase II risk assessment was not required. The risks for the environment are considered to be nil.

OVERALL CONCLUSION ON SAFETY

All accomplished investigations show that Leucofeligen FeLV/RCP is well tolerated in cats. The following local and systemic reactions were observed in the safety studies and an appropriate warning is therefore included in the SPC:

After the first injection, a moderate and transient local reaction ($\leq$2 cm) could occur (oedema, swelling, nodule). This reaction resolves spontaneously within 3 to 4 weeks at the most. After the second injection, and subsequent administrations, this reaction is markedly reduced. In rare cases, pain at palpation, sneezing or conjunctivitis may be noted, that resolves without any treatment. The transient usual signs following vaccination may also be observed, such as: hyperthermia (lasting 1 to 4 days), apathy, digestive disturbances. In case of anaphylactic shock, appropriate symptomatic treatment should be administered.

An overdose administration of Leucofeligen FeLV/RCP showed no other reaction except local reactions that can last longer (5/6 weeks at the most).
As Leucofeligen FeLV/RCP is not intended for use in pregnant animals, no study on reproductive performance has been conducted. A specific contra-indication to that effect is included in the SPC.

The genetic stability of the live virus components has been established.
The immune system of the cats is not affected negatively by using this vaccine. Leucofeligen FeLV/RCP is safe for the environment. The available data show the absence of dissemination of the Rhinotracheitis vaccine strain through the organism of the vaccinated cat and thus its inability to spread to unvaccinated animals. Even if a very low spread of Feline Calicivirus or Feline Panleucopenia virus could occur, infection of other animals with these vaccine strains could be considered as unable to induce high viral replication and disease. An appropriate warning concerning the possible excretion of FCV and FPV is included in the SPC. Serial passage in cats did not result in reversion to virulence. The studies carried out demonstrate the safety of this vaccine used at the requested minimum age of 8 weeks.

4. EFFICACY ASSESSMENT

Leucofeligen FeLV/RCP is a vaccine intended for cats only and therefore the efficacy tests have been carried out in the feline species. Leucofeligen FeLV/RCP is intended for kittens from eight weeks of age. The administration of Leucofeligen FeLV/RCP is recommended via the subcutaneous route. This route of administration was therefore used in all the laboratory and field efficacy studies. The vaccinations performed during the laboratory and field studies follow the recommended vaccination scheme.

As maternally derived antibodies (MDA) may be observed at the minimum recommended age for the first injection, especially for the FPV valence, the possible interference between vaccination and these MDA has been assessed through field studies and one laboratory study.

LABORATORY TRIALS

Onset of protection

For each component of Leucofeligen FeLV/RCP vaccine, the onset of immunity was demonstrated by challenge tests performed several weeks after the primary-vaccination of kittens, according to the corresponding Eur. Ph. monographs.

Feline Calicivirus

The efficacy of the Calicivirus valence was checked in compliance with the Eur. Ph. Monograph 1102 in a virulent heterologous challenge test performed four weeks after the primary vaccination with Leucofeligen FeLV/RCP. The clinical signs of calicivirosis and viral excretion were scored as required by Eur. Ph. Monograph. During this challenge, the scores for the vaccinated cats were significantly lower than those for the controls. These results showed that Leucofeligen FeLV/RCP reduces clinical signs of Calicivirosis, with an onset of protection of four weeks after the primary vaccination.

Feline Rhinotracheitis virus:

The efficacy of the Rhinotracheitis valence was demonstrated by a challenge trial conducted four weeks after the primary vaccination with Leucofeligen FeLV/RCP. The data related to clinical signs of the disease and viral excretion collected during these challenges was transposed to the scoring system of the Eur. Ph. monograph 1206. The scores obtained for the vaccinates were significantly lower than those for the controls. These results showed that Leucofeligen FeLV/RCP reduces clinical signs of Feline Rhinotracheitis and viral excretion with an onset of protection of four weeks after the basic vaccination scheme.

Feline Panleucopenia virus:

The efficacy of the Panleucopenia valence was checked through two challenge trials, three weeks after the basic vaccination scheme with Feligen CRP, according to the requirements of Eur. Ph. monograph 0251. During the first challenge test, the vaccinated cats remained in excellent health whereas the control cats displayed significant leucopenia. However, due to the absence of clinical signs of the disease, this study did not allow to support a claim for their reduction. Consequently, another Panleucopenia challenge was carried out. This second challenge test shows that the Panleucopenia valence of Feligen CRP, and thus of Leucofeligen FeLV/RCP vaccine, allow to prevent leucopenia and to reduce clinical signs of Feline Panleucopenia three weeks after the basic vaccination scheme. This data demonstrate that Leucofeligen FeLV/RCP vaccine prevent leucopenia and reduces clinical signs of feline panleucopenia, with an onset of immunity of three weeks after the primary vaccination.

Another study demonstrated that, when Feligen CRP is combined with Leucogen (i.e. in Leucofeligen FeLV/RCP), the humoral immunological response to FCV, FVR and FPV is similar to or higher than when Feligen CRP is administered alone. This boost of the serological response is probably due to the presence of the adjuvant in the Leucogen fraction of Leucofeligen FeLV/RCP vaccine which allows increasing the immune response. These data confirm that the results of challenge studies obtained with Feligen CRP are relevant to assess the efficacy of FCV, FVR and FPV of Leucofeligen FeLV/RCP vaccine.

Feline Leukaemia Virus:

The efficacy of the Leukaemia valence was assessed by a challenge trial, conducted in compliance with the Eur. Ph. monograph 1321 three weeks after the primary vaccination with Leucofeligen FeLV/RCP. In this study, at least 80 % of the controls were persistently infected and not less than 80 % of the vaccinated cats were protected. These results show that Leucofeligen FeLV/RCP prevent the persistent viraemia associated with a feline Leukaemia infection, and that this protection starts three weeks after the basic vaccination.

The Influence of Maternal Antibody on the Efficacy of the Vaccine

As maternally derived antibodies (MDA) may be observed at the minimum recommended age for the primary vaccination with Leucofeligen FeLV/RCP, the possible interference between vaccination and these MDA has been assessed through laboratory and field studies.

In the laboratory study, kittens born from conventional vaccinated queens received a primary vaccination with Leucofeligen FeLV/RCP (i.e. two injections of one dose at 8/9 and 11/12 weeks of age. Half of them received a third vaccine injection four weeks later (i.e.at 15/ 16 weeks of age).
Blood was collected on all cats before each injection of the primary vaccination, and two, four, six and eight weeks after the second injection.

Serological analyses showed that these kittens had undetectable or low level of MDA against FCV, FVR and FeLV viruses, but had moderate to high antibody titre to FPV at the minimum age for a primary vaccination. These observations were supported by bibliographical references and by field data. The influence of MDA on the immune response was therefore particularly considered for the FPV valence. Serological follow-ups after vaccination clearly indicate the negative influence of a high level of MDA on the immune response in kittens, and supports the efficacy of a third injection of Leucofeligen FeLV/RCP after the decline of these MDA, i.e. from 15/16 weeks of age.

This third injection was therefore recommended in the SPC, in case of expected presence of MDA.

Duration of Immunity

The duration of immunity of FCV, FVR and FeLV Leucofeligen FeLV/RCP was demonstrated by challenge tests performed one year after the primary-vaccination. The duration of immunity of Feline Panleucopenia virus component of Leucofeligen FeLV/RCP vaccine was assessed by a serological follow-up of the seroneutralising antibodies against FPV, shown to be good indicators of the protection.

Correlation of antibodies to protection for all the components (Feline Calicivirus and Feline Rhinotracheitis Virus, Feline Leukaemia Virus and Feline Panleucopenia Virus) was satisfactorily demonstrated, showing that:

- antibodies are good indicators of protection for FCV and FVR components,
- antibodies are marker of the vaccine response for FeLV component, leading to obtain a good protection rate,
- the level of antibodies is correlated with protection for FPV component.

Moreover, Leucofeligen FeLV/RCP vaccine consists of the combination of Feligen CRP and Leucogen vaccines, which are sold separately in several Member States of the European Union since 1981 and 1988 respectively. Pharmacovigilance data of these two vaccines provides supportive information on their efficacy.

<u>Feline Calicivirus:</u>

Results obtained with Feligen CRP alone are valid to demonstrate the efficacy of the same components of Leucofeligen FeLV/RCP vaccine. The duration of immunity of the Calicivirus valence of Leucofeligen FeLV/RCP vaccine was therefore shown by a heterotypic virulent challenge performed one year after the second injection of the primary vaccination with Feligen CRP. The clinical signs of calicivirosis and viral excretion were scored as required by Eur. Ph. Monograph 1102. During this challenge, the scores for the vaccinated cats were significantly lower than those for the controls, showing a one-year duration of immunity for the Calicivirus component of Feligen CRP, and thus Leucofeligen FeLV/RCP vaccine, which allows to reduce clinical signs and viral excretion.

Feline Rhinotracheitis virus:

As the results of the challenge studies performed with Feligen CRP are valid to demonstrate the efficacy of the same components of Leucofeligen FeLV/RCP vaccine, the duration of immunity of the FVR component was assessed by a virulent challenge one year after the basic vaccination scheme with Feligen CRP. The data related to clinical signs of the disease and viral excretion collected during these challenges was transposed to the scoring system of the Eur. Ph. monograph 1206. The scores obtained for the vaccinates were significantly lower than those for the controls, showing that Leucofeligen FeLV/RCP reduces clinical signs of feline rhinotracheitis and viral excretion for at least one year after the basic vaccination scheme.

Feline Leukaemia virus:

The efficacy of the Leukaemia valence was assessed by a challenge trial conducted one year after the primary vaccination with Leucofeligen FeLV/RCP. As required by the Eur. Ph. monograph 1321, at least 80 % of the controls were persistently infected and not less than 80 % of the vaccinated cats were protected. These results show that Leucofeligen FeLV/RCP prevents persistent FeLV viraemia associated with a feline Leukaemia infection, and that this protection lasts at least for one year.

Feline Panleucopenia virus:

The data provided show that one year after the basic vaccination scheme with Leucofeligen FeLV/RCP all cats presented a sufficient level of antibodies higher than the minimum protective titre.
This demonstrates a one year duration of immunity of the FPV component of Leucofeligen FeLV/RCP.

FIELD TRIALS

Clinical efficacy of the Feligen CRP/ Leucogen vaccine administered at 8/9 weeks of age

Each kitten was administered by the subcutaneous route two doses of Leucofeligen FeLV/RCP (i.e. one dose of Feligen CRP reconstituted with one dose of Leucogen) at a three-week interval (at 8/9 and 11/12 weeks of age). Clinical examinations, including weighing and rectal temperature measurement, and serological analysis were carried out on the first and the second vaccination day, and then two, three and four weeks after the second injection. The information on type of breed was presented.

The efficacy of Leucofeligen FeLV/RCP was evaluated by a seven-week serological follow-up. The sera were tested for antibodies against FCV, FVR, FPV and FeLV and the serological response was assessed by the percentages of seroconversion observed two, three and four weeks after the second vaccination. The results were analysed according to the serological status of the animals on the day of the first injection, i.e. the presence or absence of maternally derived antibodies before the vaccination.

Results:
No abnormal general or local reaction was registered after injection of Leucofeligen FeLV/RCP vaccine.
Most of the kittens were seronegative to FCV (62 %), FVR (88 %), FPV (64 %) and FeLV (96 %) prior to vaccination. High seroconversion rates were observed for the four valences in these seronegative cats.

Prior to vaccination, 38 % were seropositive for FCV, 12 % for FVR, 36 % for FPV and 4 % for FeLV. These antibodies were probably of maternal origin and could interfere with the vaccination because the seroconversion rates were lower in the seropositive animals than in the seronegative cats after the primary vaccination. The presence of maternally derived antibodies had no effect on the

seroconversion to the leukaemia valence, a moderate effect on the seroconversion to FCV, and interfered with the seroconversion to FPV and FVR.

Considering the whole population of the vaccinated cats, the maximum seroconversion rates observed after the primary-vaccination with Leucofeligen FeLV/RCP were 82 % three weeks after the second injection for FCV, 84 % three and four weeks after the second injection for FVR, 68 % four weeks after the second injection for FPV and 100 % two weeks after the second injection for FeLV.

Conclusion:
This study demonstrated that two injections of Leucofeligen FeLV/RCP vaccine induce high seroconversion rates for the four components and that a high level of maternally derived antibodies, especially those against Feline Panleucopenia Virus, can reduce this seroconversion, except for the FeLV valence. This effect of the presence of MDA on the protection, and the recommended third injection of vaccine are mentioned in the SPC (see above).

Clinical study of the efficacy and safety of a feline vaccine, Feligen CRP, under normal conditions of use

Each kitten received by the subcutaneous route a primary-vaccination with Feligen CRP (i.e. two injections of one dose at a one-month interval) and one annual booster injection (i.e. one dose of Feligen CRP one year after the second injection).

A clinical examination and blood sampling were carried out on each vaccination day, three weeks after the second and the third injections, and 3, 6, 9, 10, 11 months after the second injection.

No abnormal general or local reactions were observed after the vaccinations.

On the day of the first injection (D0), most of the kittens were seronegative against Calicivirus, Rhinotracheitis virus and Panleucopenia virus (86 %, 78 %, 55 % respectively). During the trial, the kinetics of the mean antibody titres against these three viruses in these seronegative cats can be mainly characterised by (1) a seronegative status on D0; (2) a swift seroconversion on D42; (3) a slight decrease with a persistence of a mean antibody titres at a steady level through the one-year study period; (4) a sharp increase three weeks after the annual booster vaccination.

Maternally derived antibodies against FCV, FVR and FPV were detected prior to vaccination in 14, 22 and 45 of the kittens respectively. In these seropositive cats, the kinetics of the mean antibody titres against the three viruses is similar, except for the decrease of the maternally derived antibodies between D0 and D21.

Several cats did not seroconvert to Calicivirus after the primary-vaccination. However, the sharp increase of antibody titres after the annual booster injection in these animals indicates the presence of a memory response. Thus, a cellular immune response was induced by the primary-vaccination.

A seroconversion against Calicivirus and Rhinotracheitis virus was observed after the primary-vaccination, and the antibody titres then slightly decreased during the one-year follow-up. For Panleucopenia virus, the cats seroconverted after the primary-vaccination, and the antibody titre was maintained at a high level until the annual booster. After the annual booster vaccination, a strong and rapid increase of the antibody titres was observed for the three components indicating the presence of a memory response in the vaccinated animals.

These results show that the immune response (humoral and/or cellular) induced by the primary vaccination with Feligen CRP is active for one year and strongly enhanced by the annual booster. Moreover, the titres observed after the booster injection were higher than those after the primary vaccination indicating that the cats were again protected for one year. This field study therefore confirms the conclusions of the laboratory studies on the one-year duration of immunity of Calicivirus, Rhinotracheitis virus and Panleucopenia virus contained in Feligen CRP and Leucofeligen FeLV/RCP.

This study demonstrates the duration of immunity of one year for FCV, FVR and FPV under field conditions. Furthermore, it supports the claimed vaccination scheme of two vaccinations at 3-4 weeks interval followed by an annual booster vaccination.

During the second study, cats received a primary vaccination (i.e. two injections of one dose at a one-month interval) and one annual booster injection with Leucogen vaccine (i.e. one dose of Leucogen one year after the second injection). Each cat received by the subcutaneous route two injections of one dose of Leucogen (antigen content: 108 µg/ml) at a three-week interval (primary-vaccination) and a third injection of the same vaccine one year later (annual booster injection). All the kittens were also vaccinated with Feligen CRP at the same time but at different injection sites.

A clinical examination and blood sampling were carried out at the vaccination times (W0, W3 and W55), three and four weeks after the primary o-vaccination (W6 and W7) and three weeks after the annual booster (W58). The serum was tested for the presence of anti-p45 antibodies. The body weight was measured on three occasions (on W–1, W7 and W55). The serum was not tested for FCV, FVR and FPV, but the fact that the vaccine had been administered allowed to evaluate the impact of the said antigens on the immune response to the FeLV component of Leucogen. The information on type of breed was presented.

All the cats seroconverted to P45 protein after the primary-vaccination, and many of them were still seropositive one year later. All of them recovered high antibody titre three weeks after the annual booster, indicating the presence of a memory response during at least one year following the basic vaccination scheme.

Results:
Few mild and transient local reactions were observed.
Thirty-nine kittens were chosen for analyses after the primary-vaccination. Seroconversion against FeLV was seen in 27 animals (69 %) after the first injection and in 100 % after the second injection. Only one kitten presented maternally derived antibodies to FeLV prior to the vaccination. After vaccination, this cat seroconverted similarly to the others, thus indicating that the presence of maternally derived antibodies did not interact with the serological response to Leucogen.

Twenty-eight cats were chosen for analyses after the annual vaccination (W55). None of the cats showed local or abnormal general reactions after this last injection. Eighteen cats (64 %) still showed anti-p45 antibodies before the annual booster injection. Three weeks after the booster injection (W58), all cats showed high level of antibodies against p45.

Conclusion:
This study demonstrates that Leucogen vaccine induces high and long-lasting antibody titres to FeLV, which were not affected by Feligen CRP administered at the same time. The strong and rapid enhancement of the serological response to the annual booster vaccination revealed the presence of a memory response one year after the primary-vaccination. These results therefore confirm the one-year duration of immunity of Leucogen and of the FeLV component of the Leucofeligen FeLV/RCP. This study demonstrated the duration of immunity of one year for FeLV under field conditions. It supported the claimed vaccination scheme of two vaccinations at a 3-4 weeks interval followed by an annual booster vaccination.

Pharmacovigilance as supportive data for efficacy:
Leucofeligen FeLV/RCP consists of two vaccines (Feligen CRP and Leucogen) that have been granted Marketing Authorisations in several European countries since 1981 and 1988 respectively. The two vaccines have proven to be effective for more than fifteen years of use in the field. The Pharmacovigilance reports presented show the efficacy of Feligen CRP and Leucogen used separately and complete the field study.

OVERALL CONCLUSION ON EFFICACY

In order to demonstrate efficacy of Leucofeligen FeLV/RCP several studies have been performed by the Applicant.

Onset of immunity:
- FCV-Challenge
- FVR-Challenge
- FPLV-Challenge
- FeLV- Challenge

Duration of immunity:
- FCV-Challenge
- FVR-Challenge
- FPLV-Serology
- FeLV-Challenge

Efficacy of the vaccine in the presence of maternally derived antibodies:
-Laboratory study (serology only)
-Serological data of the field study

Field study
Three field studies were conducted to verify the immunisation scheme of Leucofeligen FeLV/RCP.

Vaccination with Leucofeligen FeLV/RCP on two occasions at intervals of 3-4 weeks in kittens, aged 8 weeks or older, reduces clinical signs caused by an infection with FCV, reduces clinical signs and viral excretion caused by an infection with FVR, prevents Leucopenia, reduces clinical signs caused by FPV and prevents persistent viraemia and clinical signs of the related disease caused by a FeLV infection.
The onset of immunity has been demonstrated by challenge to be three weeks after the primary vaccination for the Panleucopenia and Leukaemia components, and four weeks after the primary vaccination for the Calicivirus and Rhinotracheitis virus components.

The duration of immunity is one year after the primary vaccination for all components, demonstrated by challenge for FCV, FVR and FeLV and by serology for FPV. The studies were carried out with cats of minimum age and with low titres of the viral components. The experimental setup and the results of the studies performed by the Applicant are acceptable.

Protection in the face of maternally derived antibodies has been shown by serology during field study and one laboratory study. These studies demonstrated the possible influence of MDA on the vaccination against FCV, FVR and FPV. Therefore, the Applicant states the following in the SPC:

Section 4.4: “Maternally derived antibodies, especially those against Feline Panleucopenia Virus can negatively influence the immune response to vaccination.”
Section 4.9: “Maternally derived antibodies can negatively influence the immune response to vaccination. In such cases, where maternally derived antibodies are expected, a third injection may be appropriate from 15 weeks of age.”

Based on the data presented by the Applicant, the following indication for use of the vaccine is justified:

For active immunisation of cats from eight weeks of age against:

- feline calicivirosis to reduce clinical signs
- feline viral rhinotracheitis to reduce clinical signs and viral excretion
- feline panleucopenia to prevent leucopenia and to reduce clinical signs
- feline leukaemia to prevent persistent viraemia and clinical signs of the related disease.

Onset of immunity: 3 weeks after the primary vaccination for the panleucopenia and leukaemia components, and 4 weeks after the primary vaccination for the calicivirus and rhinotracheitis virus components.

The duration of immunity is one year after the primary vaccination for all components.

The proposed vaccination scheme is as follows:

Primary vaccination :
- first injection in kittens from 8 weeks of age
- second injection 3 or 4 weeks later.

Revaccination:
Annual

Section 4.4: "Maternally derived antibodies, especially those against Feline Panleucopenia Virus can negatively influence the immune response to vaccination."
Section 4.9: "Maternally derived antibodies can negatively influence the immune response to vaccination. In such cases, where maternally derived antibodies are expected, a third injection may be appropriate from 15 weeks of age."

5. BENEFIT – RISK - BALANCE

It should be noted that the products Leucogen and Feligen RCP have already been on the market for more than 10 years.

Leucogen, already sold for many years, world-wide, and in particular in several countries of the European Union.
Leucofeligen FeLV/RCP contains three live attenuated viral strains (FCV, FVR and FPV) and a purified p45 recombinant protein derived from the gp70 surface glycoprotein of the FeLV. The final association includes aluminium hydroxide and highly purified extract of *Quillaja saponaria.* The vaccine consists of a lyophilisate: Feligen RCP and solvent for suspension for injection, Leucogen. Leucogen, is a highly purified vaccine against feline leukaemia produced by genetic engineering. Sequence of the envelope protein of FeLV was introduced via a plasmid in an appropriate *Escherichia coli*. FeLV envelope p45 protein is initially expressed in large quantities in inclusion bodies. These bodies are extracted and thoroughly purified. The final preparation includes the addition of aluminium hydroxide and highly purified fraction of saponin. It is presented in vials containing one dose of 1ml as liquid preparation.

The description of the construction of the plasmid and its insertion into the E.coli vector is clear and comprehensive. Identity, purity and genetic stability of the seed are well demonstrated. The characteristics and the controls of the purified p45, also tested in accordance with the various pharmacopoeial and guideline requirements, are fully documented as well. Since the vaccine itself is highly purified and does not contain retraceable DNA, there are no concerns regarding potential recombination or reassortment.

All other starting materials, including those of biological origin are extensively tested and have been shown to be of suitable quality. With regard to the risk of transmission of spongiform encephalopathy,

the quality of starting materials of biological origin is in compliance with the current regulations on managing the TSE transmission risk. The overall TSE risk of the vaccine is therefore practically nullified.

The Leucofeligen FeLV/RCP production process is carefully controlled. Appropriate tests are applied throughout the procedure ensuring batch quality and consistency at every stage. Production process related consistency is documented convincingly. A rational update of the batch potency test taking into account the historical experience in the handling of the product is presented with a thorough validation.

The shelf-life of 24 months is justified. The products is manufactured and tested within a high level of GMP and Quality Assurance system, which ensures batch to batch consistency.

Leucofeligen FeLV/RCP contains three live attenuated viral strains (FCV, FVR and FPV) and a purified p45 recombinant protein derived from the gp70 surface glycoprotein of the FeLV.

As Panleucopenia and Leukaemia wild viruses are known to induce immunosuppression, the effect of the corresponding vaccinal components on immunological functions were assessed (i.e. the panleucopenia vaccinal strain and the p45 recombinant protein). Vaccination using Leucofeligen FeLV/RCP has no negative impact on the immunological function of cats. The requirements of the monographs are fulfilled satisfactorily. Studies have been performed to analyse the dissemination of the vaccine strains in the predilection sites for replication. They showed the very restricted replication of the Calicivirus vaccinal strain and the absence of dissemination of the Rhinotracheitis virus vaccinal strain in the target respiratory organs. Although the Panleucopenia vaccinal strain was detected in the faeces, this strain does not show any typical signs of virulence of the parvoviruses. It has been demonstrated that Feline Calicivirus and Feline Panleucopenia Virus vaccinal strains can spread but did not cause adverse reactions on non-vaccinated cats. The corresponding studies demonstrate the absence of reversion to virulence of Feline Calicivirus strain, Feline Rhinotracheitis strain and Feline Panleucopenia strain. No severe adverse reactions occurred after administration of a single dose, an overdose or repeated doses of Leucofeligen FeLV/RCP.

In summary, the benefits of Leucofeligen FeLV/RCP are for feline calicivirosis the reduction of clinical signs, for feline viral rhinotracheitis the reduction of clinical signs and viral excretion, for feline panleucopenia the prevention of leucopenia and reduction of clinical signs, and for feline leukaemia the prevention of persistent viraemia and clinical signs of the related disease. The most common side effects are a moderate and transient local reaction ($\leq$ 2cm) which can occur after the first injection but which resolves spontaneously within 3 to 4 weeks at the most. After the second injection, and subsequent administrations, this reaction is markedly reduced. In rare cases, pain at palpation, sneezing or conjunctivitis may occur which resolve without any treatment. Transient signs such as hyperthermia, apathy and digestive disturbances may also be observed following vaccination.

The approved indication is: “For active immunisation of cats from eight weeks of age against:

- feline calicivirosis to reduce clinical signs.
- feline viral rhinotracheitis to reduce clinical signs and viral excretion.
- feline panleucopenia to prevent leucopenia and to reduce clinical signs.
- feline leukaemia to prevent persistent viraemia and clinical signs of the related disease.”

The CVMP, on the basis of quality, safety and efficacy data submitted, considers that there is a favourable benefit to risk balance for Leucofeligen FeLV/RCP and therefore recommends the granting of the marketing authorisation.

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