SCIENTIFIC DISCUSSION

Invented Name:	Purevax RCP FeLV
Active substance / INN:	Attenuated feline rhinotracheitis herpesvirus (FVH F2 strain): at least 4.9 log 10 CCID50 per dose Inactivated feline calicivirosis antigens (FCV 431 and FCV G1 strains): at least 2.0 ELISA U per doses Attenuated feline panleucopenia virus (PLI IV): at least 3.5 log 10 CCID50 per dose FeLV recombinant canarypox virus (vCP97): at least 7.2 log 10 CCID50 per dose
Target species:	Cats
Therapeutic indication:	Active immunisation of cats of 8 weeks of age and older: - against feline infectious rhinotracheitis to reduce clinical signs, - against calicivirus infection to reduce clinical signs and excretion, - against feline panleucopenia to prevent mortality and clinical signs, - against leukaemia to prevent persistent viraemia and clinical signs of the related disease.
Withdrawal period:	Not applicable
Pharmaceutical form:	Lyophilisate and solvent for suspension for injection
ATCvet code	QI
Pharmaco-Therapeutic Group	Immunologicals
Marketing Authorisation Holder	Merial 29 avenue Tony Garnier 69007 LYON France

1. SUMMARY OF THE DOSSIER

Purevax RCPCh FeLV is a non-adjuvanted vaccine against feline viral rhinotracheitis (modified live feline herpesvirus F2 strain), calicivirosis (combination of two inactivated purified viruses, FCV431 and FCVG1 strains), infectious panleucopenia (modified live parvovirus, PLI IV strain) and feline leukaemia (recombinant canarypox-FeLV, vCP97 strain).

The vaccine consists of a lyophilisate containing the active ingredients of feline viral rhinotracheitis, calicivirosis and infectious panleucopenia to be reconstituted with a solvent containing the recombinant canarypox-FeLV active ingredient.

The claims of the vaccine are:
Active immunisation of cats of 8 weeks of age and older:

- against feline infectious rhinotracheitis to reduce clinical signs,
- against calicivirus infection to reduce clinical signs and excretion,
- against feline panleucopenia to prevent mortality and clinical signs,
- against leukaemia to prevent persistent viraemia and clinical signs of the related disease.

The vaccination schedule recommends the subcutaneous injection of a first dose of 1 ml of vaccine from the age of 8 weeks. Three to 4 weeks later, a second dose of 1 ml of vaccine is injected. Annual booster vaccination is recommended for all components except for feline panleucopenia (vaccination every 3 years after the first annual booster).

2. QUALITY ASSESSMENT

Composition

Active substances:
Per 1 ml dose:
Freeze-dried pellet:
Attenuated feline rhinotracheitis herpesvirus (FHV F2 strain) ..... $\geq 10^{4.9}$ $CCID_{50}$[1]
Inactivated feline calicivirosis antigens (FCV 431 and G1 strains) ..... ≥ 2.0 ELISA U.
Attenuated feline panleucopenia virus (PLI IV) ..... $\geq 10^{3.5}$ $CCID_{50}$[1]
Excipient:
Gentamicin .....at most 34 µg
Solvent:
FeLV recombinant canarypox virus (vCP97) ..... $\geq 10^{7.2}$ $CCID_{50}$[1]

[1]: cell culture infective dose 50%
[2]: egg infective dose 50%

Container

Bottle (freeze-dried pellet or solvent) closed with an elastomer-derived closure and sealed with an aluminium cap.

Development Pharmaceutics

The development of Purevax RCP FeLV addressed the 4 major features of the vaccine. These are the absence of an adjuvant, the inclusion of a new calicivirus antigen, the demonstration of the efficacy and

safety of the canarypox-FeLV recombinant virus as well as the fact that Purevax RCP FeLV is one of the products of a complete line with several combinations allowing veterinarians to adapt the vaccination programme to the needs of the cats depending on their environment and way of life. This is an efficient way to increase flexibility of use and to avoid over-vaccination. This contributes to a better safety of vaccination in the feline species.

The choice of both the vaccine strains and the antigen quantification method was adequately demonstrated. The formulation was demonstrated to be the best suited pharmaceutical form for a live virus vaccine. The overage, release specifications and formulation targets were satisfactory for this type of vaccine.

Method of manufacture

The different stages of production (formulation, filling, freeze-drying and packaging) were described in a detailed manner. All the operations are carried out in closed circuit, except for the transfer into the vessels of the active ingredients thawed in a waterbath initially set at 37°C (± 3°C) which is carried out under laminar air flow hood (grade A) in clean, contained production areas of grade B (Good Manufacturing Practice (GMP) classification). All connections are sterilised by steam or gamma-radiated. When sterilisation operations are described, these are carried out in compliance with the current edition of the European Pharmacopoeia.

CONTROL OF STARTING MATERIALS

Active substance

The active ingredients used in the production of the veterinary vaccine, the attenuated feline herpesvirus, the inactivated feline calicivirus 431 antigen, the inactivated feline calicivirus G1 antigen, the attenuated feline panleucopenia virus and the vCP97 virus were adequately characterised, their origin detailed and the processing explained.

Genetic engineering
vCP97 is an ALVAC-based recombinant virus containing the feline leukaemia virus subgroup A *env* and *gag* genes and a portion of the *pol* gene. The canarypoxvirus Rentschler strain was isolated in Germany in 1970, attenuated in order to obtain the vaccine strain used in another veterinary vaccine. This vaccine strain was amplified to obtain the vector referred to as ALVAC. The feline leukemia virus subgroup A strain Glasgow-1 was chosen as donor organism

The *env* gene, under the control of the promoter H6, was inserted by a first recombination. The resulting virus was cloned by the method of plaques, with satisfactory homogeneity of the clone. The *gag* gene and the portion of the *pol* gene, under the control of the same promoter H6, was inserted by a second recombination. The resulting virus was cloned to obtain a clone called vCP97. The arguments supplied concerning the purity of the clone are satisfactory. Data on the boundaries of the inserted sequences are detailed. The vCP97 clone has again been purified by the method of plaques. The expression of the FeLV *env* and *gag* genes was demonstrated using immunological methods.
The genetic stability was demonstrated by showing the absence of modification after 5 sequential passages.

Excipients

The substances of biological origin used in the production of the veterinary vaccine, lactalbumin hydrolysate, calf serum, trypsin, foetal calf serum, Pronase, casein hydrolysate, collagen hydrolysate and

tryptase phosphate broth (TPB) were adequately characterised, their origin detailed and the processing explained and compliance with the relevant Ph. Eur.monographs demonstrated.

All starting materials of non-biological origin were listed and satisfactory certificates of analysis provided.

Qualitative and quantitative compositions of all media were provided. The media, after undergoing a prefiltration step, were sterilised by filtration through a membrane of nominal pore size ≤ 0.22 µm. Filter integrity tests are carried out.

Packaging

All packaging material was of a satisfactory nature.

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

This vaccine is intended for cats. Cats and other felidae have been shown to be susceptible to TSE when exposed to infectivity by the oral route. However, as cats are domestic animals there is no discernable risk of spreading the disease.

Lactose monohydrate, Lactalbumine hydrolysate and Casein hydrolysate
The milk derivatives are considered to be in compliance with the NfG provided that the milk is sourced from healthy animals in the same conditions as the milk collected for human consumption and that the calf rennet, if used during the preparation of the lactose, complies with the public statement on lactose prepared using calf rennet of the EMEA/CPMP/571/02.

Tryptose Phosphate Broth
The milk used in tryptose phosphate broth is sourced from healthy animals and the applicant confirmed that the milk is fit for human consumption and that no rennet is used.

F10-199 medium
The F10 medium does not contain any component of animal origin. The 199 medium contains cholesterol from New Zealand sheep wool and does not contain any other material of animal origin. No cases of scrapie have been reported in New Zealand. It is considered by the “Note for Guidance on minimizing the risk of transmitting TSE agents via human or veterinary medicinal products" that wool derivatives are in compliance with this Note for Guidance provided the wool is sourced from live animals.

Calf serum, Foetal calf serum
Copies of EDQM certificates were provided for all mentioned suppliers except one, for which a scientific dossier was provided.

Collagen hyrolysate
A copy of the EDQM certificate has been provided.

CrFK cells (Crandell Feline Kidney cell line)
MDCK cells (Canine kidney cell line: Madin-Darby Kidney Cell Line)
IRC5 cells (Cat kidney line cells Iffa Rein de Chat 5)
Attenuated Feline herpesvirus
Inactivated Feline Calicivirus 431 antigens
Inactivated feline calicivirus G1
Attenuated Feline Panleucopenia virus
VCP97 virus

The origin of all these cell lines and viruses was described in satisfactory detail and any safety issues relating to the potential TSE risk were adequately addressed.

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 Directive 2001/82/EC.

CONTROL TESTS DURING PRODUCTION

The specific tests carried out during the production of the active ingredients were described.
The other tests concerned formulation, filling and packaging. They included

- time recording
- temperature recording
- viral purity
- monitoring of the sterilisation cycle (compliant with Ph.Eur., 5.1.1),
- checking of the filled volume,
- checking of the freeze-drying cycle,
- checking of the appearance of the product after capping,
- checking of the appearance of the product after labelling,
- checking of the appearance of the product presentation after packaging,
- checking the filter integrity in compliance with Ph. Eur. 5.1.1.

and while no certificate exists for these tests, they will be present in the batch record.

CONTROL TESTS ON THE FINISHED PRODUCT

General tests included the appearance and the pH of the lyophilisate and the solvent, as well as the volume and the compatibility of the solvent.

Stability of the finished product

Lyophilisate
The study was carried out on three batches of RCPCh freeze-dried pellet. Results show the stability of the vaccine after a storage period of at least 21 months at 5°C ± 3°C in bottles protected from light. The physico-chemical parameters (pH, residual humidity) remain stable during the 21 months storage period. The bacterial, fungal and mycoplasmic sterility, as well as the safety is demonstrated after 27 months of storage. Titrations are carried out at different times during the storage period. The titres of each valence remain within the specification after 21 months of storage. The average loss of titre can be estimated after 21 months of storage to be - 0.30 log10 CCID50 for the FHV component, - 0.10 ELISA units for the FCVcomponent and not significant for the PLI component. Based on the results obtained, a 18-month shelf life is justified at 5°C ± 3°C in bottles protected from light.

Solvent
The relevant study was carried out on three batches of solvent containing the vCP97 component. Intermediate results show the stability of the vaccine after a storage period of 27 months at 5°C ± 3°C in bottles protected from light.

The physico-chemical parameters (pH, residual humidity) remain unchanged during the storage period. The bacterial and fungal sterility is demonstrated after 15 and 27 months of storage, except for one batch at T27 months. Specific safety is demonstrated after 27 months of storage. Titrations are carried out at different times during the storage period. The average loss of titre can be estimated after 27 months of storage to be - 0.29 log10 CCID50/ml.

Consequently, a shelf-life of 24 months at 5°C ± 3°C in bottles protected from light is justified for the solvent.

A stability study was carried out on the reconstituted vaccine stored at 22°C for 2 hours. This study was carried out on three batches. On the one hand, it was intended to evaluate the stability of the reconstituted vaccine during 2 hours at 22 °C ±3 °C, on the other hand, it is also intended to evaluate the possible virucidal activity of the solvent on the components of the freeze-dried pellet: FHV, FCV and PLI.
The general conclusions were:

- The stability of the reconstituted product is very good for FHV, FCV and PLI and the vaccine can be kept for 2 hours after reconstitution at + 22°C ± 3°C, protected from light.
- The virucidal effect of the FeLV solvent is absent for FHV and PLI.

Recombinant technology

The vCP97 construct does not show any documented risk and no foreseeable risk associated with its use can be identified. Major arguments are:

<u>The ALVAC is a vector of choice in terms of safety</u>:
The canarypox virus has a narrow host range. Several veterinary vaccines have been registered in North America (canarypox-Rabies for cats, canarypox-Distemper for dogs and ferrets) and Europe (canarypox-FeLV, canarypox-equine Influenza) and several ALVAC constructs are currently under investigation in human trials (cancer, HIV, malaria…) and some of them are included in phase 2 trials. The biosafety related to the absence of multiplication in mammals has been extensively confirmed in field conditions and in various species.

<u>The safety of the vCP97 construct has been confirmed</u>:
The vCP97 construct does not multiply in mammalian cells. In the canary, the natural host, vCP97 is safe and replication and persistence are very low. Spreading is limited to canaries kept in close contact. In the cat, vCP97 is safe and does not spread after vaccination.
The vaccine is manufactured and delivered in tightly closed vials. It is directly and individually injected to each cat via the subcutaneous route only by a veterinary surgeon, limiting thereby the contact with the environment.

In conclusion, the CVMP considered that the biosafety of the vCP97 was adequately addressed.

OVERALL CONCLUSION ON QUALITY

The analytical part is generally well documented. The production and control of starting materials follows the recommendations of the EU note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01-Rev.1).

The Applicant nevertheless provided the following commitments relating to Part 2 of the dossier.

1) As the CVMP decided that the sizes of the batches used in the validation study were not adequate for the proposed production of up to 200000 vials of lyophilisate the Applicant committed to limit the production to 80 000 vials. In the case that the Applicant intends to scale up its production from 80 000 to 200 000 vials the Applicant will apply for a variation.
2) The Applicant committed to placing the first two manufacturing scale batches into a long-term stability programme after the granting of the Marketing Authorisation.
3) The Applicant committed to use gentamicin sulphate, lactose monohydrate, sodium chloride and sorbitol which comply with the new Ph. Eur. monographs.
4) The Applicant committed to send all the final experiment reports corresponding to the stability studies of the active ingredients as soon as they are finalised.
5) Regarding the validation of the titration of feline panleukopenia the Applicant committed to validate technique No.15 351 in the combined vaccine according to a strict timetable.

As for the part 2H, the environmental risk assessment for the genetically modified canarypox virus has been satisfactorily addressed, taking into account the remarks that were expressed during the environmental assessment of that same GMO used as a component of two previously assessed centrally authorised vaccines.

3. SAFETY ASSESSMENT

A major feature of Purevax RCP FeLV is the absence of adjuvant. Tolerance at the injection site is of great importance in feline vaccinology. It was, therefore, decided to develop an adjuvant-free vaccine so as to limit the inflammatory reaction at the injection site. As a consequence of the absence of adjuvant, most components of the vaccine are modified live agents (feline viral rhinotracheitis, infectious panleucopenia) or vectored virus (canarypox-FeLV), with the exception of the feline calicivirus component, which has been inactivated.

Importantly, Purevax RCP FeLV contains 3 components which have already been registered and widely used either in North America (feline viral rhinotracheitis) or in Europe (infectious panleucopenia and feline leukaemia components). The only new component is the inactivated calicivirus antigen combination.

The safety studies have not been carried out using the vaccine under application Purevax RCP FeLV but using the vaccine Purevax RCPCh FeLV. This vaccine contains the same components as Purevax RCP FeLV and one additional component, a modified live *Chlamydophila felis* against feline chlamydiosis. This safety approach is acceptable and in line with the Note for Guidance CVMP/IWP/52/97-FINAL "Requirements for combined veterinary vaccines", as stated in section 3. Safety Aspects: "safety tests carried out on the combined vaccines may be regarded as sufficient to demonstrate the safety of the individual components or vaccines containing a smaller number of components providing the components (antigens, composition of excipients and/or adjuvants) are identical in each case and it is only the number of active ingredients which is changed".
Consequently all references to studies indicated in the safety assessment will refer to Purevax RCPCh FeLV and references to the Chlamydia component will only be deleted were appropriate.

The safety trials were carried out in the target species i.e. cats, in both young and adult animals. Some of the trials were carried out in other species (minks, dogs, canaries, chickens, mice and guinea-pigs) in order to provide further evidence of the safety of the feline panleucopenia and the recombinant canarypoxvirus components.

The vaccine dose administered to the cats corresponded to that recommended in the instructions for use.

The batches of vaccines used in the trials were produced in accordance with the manufacturing process, described in the analytical dossier, except for the FeLV component that was freeze-dried instead of being included in the solvent. This had no impact on the conclusions of the concerned studies, as both vaccines have very similar compositions.

For some trials, vaccines containing the same vaccine strains as Purevax RCPCh FeLV, but not systematically all together, were used. These vaccines are called: Feliniffa™ (P component), Eurifel FeLV (FeLV component) and Eurifel RCChPFeLV (HCChP and FeLV components). Vaccines containing equivalent components, but from vaccine strains different than those present in the Purevax RCPCh FeLV vaccine have been used in some trials in order to allow comparisons with the Purevax RCPCh FeLV vaccine: Competitor Product A (FeLV component) and Competitor Product B (HCChP components).

Purevax RCPCh FeLV is an associated vaccine containing live and inactivated components: a modified live feline herpesvirus (F2 strain), a combination of two inactivated purified caliciviruses (FCV431 and FCVG1 strains), a modified live *Chlamydophila felis* (strain 905), a modified live infectious panleucopenia virus (strain PLI IV) and a recombinant canarypox-FeLV (strain vCP97) against feline leukaemia. The vaccine does not contain any adjuvant.

While the safety studies were not conducted with the final product since the FeLV component was included in the lyophilisate and not in the liquid form, this is not considered to be a safety concern. No

study on the safety of the administration of one dose was specifically conducted. The Applicant referred to a combined study on the safety of the administration of an overdose followed by two repeated administrations of one dose.

For the administration of an overdose, the titres administered for the FeLV component and for the Ch component were below 10 times the maximum release titre. These deficiencies were covered by other studies where maximal titres of these components were individually administered.

Concerning the repeated administration of one dose, the titres administered for the C, the FeLV, and the Ch components were below the maximum release titre but these deviations were satisfactorily justified.

The results of the overdose shows transient hyperthermia occurring 4 hours after the injection and lasting from 24 to 48 hours, exceptionally 5 days. Hyperthermia was still observed after 72 hours in 50% of the animals. No other systemic reactions were observed. Local reactions at the injection site were transient pain and swelling. A significant but transient decrease in the number of circulating leukocytes was frequently observed.

Following the repeated administration of one dose, transient hyperthermia was rarely observed. Local reactions were transient pain and swelling. Repeated injections do not amplify the adverse effects already observed following the administration of an overdose. A significant but transient decrease in the number of circulating leukocytes was frequently observed after the second injection.

A study was carried out to evaluate local safety of the Purevax RCPCh FELV vaccine by histology of the injection site. In all injection samples, only minimal to moderate reactions could be observed. On two occasions, no reaction could be detected on histological examination. In conclusion, the local safety of the non adjuvanted combined Purevax vaccine, administered by subcutaneous route was good and the reactions at injection site were mild to moderate. No specific study has been carried out to evaluate the impact on reproductive performance. The SPC adequately recommends not to vaccinate pregnant animals.

The examination of immunological functions was focused on the leukopenic properties of panleucopenia and leukaemia components. Concerning specific immunity, histological examination of the thymus showed hypoplasia with a decrease in the number of cells of the cortical substance. This effect is not unexpected with a live panleucopenia vaccinal strain. A specific study showed that immunological function was not affected by vaccination with a high dose of panleucopenia and canarypox-FeLV.

The feline herpesvirus F2 strain seems not to disseminate in cats. However the absence of spreading has not been confirmed. Given the narrow host range susceptibility to FHV-1, no safety concern to species other than felidae is expected. Due to the high genetic stability of FHV and to the absence of spread of the vaccine virus, the risk of recombination between the FHV F2 strain and any wild type strain or different vaccine strains would be negligible.

The feline panleucopenia virus vaccine strain is excreted mainly in the faeces of the vaccinated cats. The dissemination in the body has not been studied. No reversion to virulence has been observed comparing a 6^{th} passage to the vaccinal strain. The safety for minks and dogs has been demonstrated. Parvovirus vaccines are rated safe and genetically stable.

The *Chlamydophila felis* vaccinal strain has been shown to disseminate to ocular secretions. However, spreading to naïve cats was not observed. No reversion to virulence was demonstrated in a first series of passages. Recombination and genetic reassortment is not expected to be of concern.

Given the non-replicative properties of the recombinant canarypoxvirus, no spread, dissemination and reversion to virulence is expected. The safety for canaries, chicken, guinea-pigs and mouse has been demonstrated. No interaction study is presented. This is reflected in the SPC.

Two field trials were conducted including 115 cats of different age and breed. They were carried out with the Purevax RCPCh FeLV vaccine at maximum potency or at potency close to maximum for all the components except for *Chlamydophila felis* and this was adequately justified.

The general reactions observed were apathy and/or anorexia, rarely hyperthermia. In most cases they were transient (less than two days). It should be noted that lethargy and apathy observed in the field were not recorded in laboratory trials. These reactions are, however, not unexpected and were therefore mentioned in the SPC. The local reactions were pain at injection site and swelling, as already observed in laboratory trials.

A main deficiency in the field trials was that no kittens of the minimal recommended age of 8 weeks have been vaccinated. The Applicant, therefore, performed a new field study including kittens and cats ranging from 6.4 weeks to 16.3 years. A significant number of kittens (n = 78) aged 1.5 – 3 months were included. The vaccine used was a commercial formulation containing the vaccinal valences at intermediate potency. No other reactions were seen as in the laboratory studies and the previous field studies, except for a slight shock reaction in one cat. This study confirmed the safety of the vaccine under field conditions.

No potential risk for the environment is expected.

4. EFFICACY ASSESSMENT

Purevax RCP FeLV is a non-adjuvanted vaccine against feline viral rhinotracheitis (modified live feline herpesvirus F2 strain), calicivirosis (combination of two inactivated purified viruses, FCV431 and FCVG1 strains), , infectious panleucopenia (modified live parvovirus, PLI IV strain) and feline leukaemia (recombinant canarypox-FeLV, vCP97 strain).

The Purevax vaccines have been developed to offer a non-adjuvanted vaccine range affording a satisfactory protection after a classical primary vaccination with two administrations and one annual booster (less if feasible). This has primarily driven the choice towards live components (except for calicivirus).

The valences of the vaccine are those recommended for the primary vaccination of kittens. FHV-1 is responsible for feline viral rhinotracheitis, a respiratory and ocular disease and FCV is responsible for acute and chronic gingivo-stomatitis. These two agents are commonly associated in a syndrome called "feline coryza", occurring at a high incidence in all cat populations. FPLV is the aetiologic agent of panleucopenia, a generalised lethal disease of young cats. FeLV infection is associated with various diseases, including immune depression, lymphosarcoma, lymphoid and myeloid leukaemia, infertility and anaemia. The composition of the vaccine is, therefore, most relevant with regard to these high incidence viral pathologies in the cat.

The trials have been conducted both in the laboratory and in the field, using the recommended route of administration (subcutaneous).

The efficacy studies have not been carried out using the vaccine under application Purevax RCP FeLV but using the vaccine Purevax RCPCh FeLV. This vaccine contains the same components as Purevax RCP FeLV and one additional component, a modified live *Chlamydophila felis* against feline chlamydiosis.
This efficacy approach is in line with the Note for Guidance CVMP/IWP/52/97-FINAL "Requirements for combined veterinary vaccines", as stated in section 4. Efficacy Aspects: "test results from large combinations should be acceptable for smaller combinations of the same antigens … providing the components (antigens, composition of excipients and/or adjuvants) are identical in each case and it is only the number of active ingredients which is changed". The presence of additional *Chlamydophila felis* component is very unlikely to induce synergistic interactions as it is not antigenically related to one of the other components.
Consequently all references to studies indicated in the efficacy assessment will refer to Purevax RCPCh FeLV and references to the Chlamydia component will only be deleted were appropriate.

The tests were carried out in accordance with the requirements in force at the time of their implementation and enabled the evaluation of the efficacy of Purevax RCPCh FeLV under the proposed conditions of use.
The requirements of Directive 2001/82/EC concerning efficacy trials in live and inactivated vaccines were met. For feline leukaemia component, the European Pharmacopoeia monograph, even if not fully appropriate (inactivated vaccine, Vaccinum leucosis felinae inactivatum), was used as a guide. For feline viral rhinotracheitis, calicivirus and panleucopenia components, the efficacy was demonstrated in accordance with Eur. Ph. monographs No.1206, 1101 and 0251 respectively.

Purevax RCPCh FeLV vaccine contains the vCP97 live recombinant virus, as Eurifel FeLV and Eurifel RCPFeLV. In accordance with Directive 90/219/EC on the contained use of genetically modified micro-organisms, appropriate authorisations were obtained in France for the contained use of this component.

With regards to deliberate use of vCP97 in Purevax RCPCh FeLV vaccine, appropriate authorisation was obtained in France in accordance with Directive 90/220/EC on the deliberate release into the environment of genetically modified organisms.

The choice of the vaccinal strains was considered appropriate.

All the efficacy trials were carried out in the feline species, in young and adult animals. The dose used in the efficacy trials had a volume of 1 ml (claimed quantity for a dose of this product). Vaccines were administered via the recommended subcutaneous route of administration, according to the schedule of vaccination.

The efficacy of the vaccine in conventional kittens (as opposed to SPF kittens) of the minimal recommended age for vaccination has not been investigated considering the difficulties to perform adequate serological and challenge studies in young conventional kittens. This can be considered as justified regarding the data provided on the kinetics of maternally derived antibodies (MDAs). Indeed for kittens the difference between SPF animals and conventional ones is the presence of MDAs. The Applicant provided data showing that for most components the MDA had disappeared and/or decreased to very low level for FHV and FCV. For *Chlamydophila felis* low titres were seen at 10 weeks. The main concern is FPV, for which interference with MDA is well known. Therefore the sentence "in the presence of high level of MDA, the primary vaccination course should be delayed until 12 weeks of age" has been added in the updated SPC.

Protection against feline infectious rhinotracheitis

The determination of the minimal protective dose for the herpesvirus component was supported by two studies. Firstly, a study showed that the FHV F2 strain administered at a titre of at least 5.5 $\log_{10}$ $CCID_{50}$ in 10 week old cats reduces clinical signs and viral excretion against a FHV challenge performed 8 weeks after vaccination. In the second study, vaccination of 8 week old cats with Purevax RCPCh FeLV vaccine at a titre of 4.9 log 10 $CCID_{50}$ FHV provided a significant reduction of clinical signs against challenge performed 4 weeks after vaccination. Although the duration of excretion was usually reduced in vaccinates, the difference in global excretion between the vaccinates and controls was not statistically significant. Therefore, the present data do not support the claim of reduction of excretion of FHV.

The duration of protection against infectious rhinotracheitis was demonstrated for a vaccinal dose of $10^{5.3}$ $CCID_{50}$ FHV F2 that induced a significant protection against challenge carried out more than one year after second injection of primary vaccination. This protection consisted of a reduction of clinical signs and global viral excretion. Using a vaccinal dose of 5.0 log10 CCID50 FHV F2 only reduction of viral excretion was observed. However in another study, the Purevax RCPCh FeLV vaccine at a titre of 5.0 log10 CCID50 per dose (slightly higher than the minimum recommended 4.9 log10 CCID50) for the herpesvirus component induced a significant reduction of general and local symptoms, and of global virus shedding after a herpesvirus challenge.

Considering all the data provided, the claim of reduction of clinical signs of feline rhinotracheitis is demonstrated. On the other hand the reduction of viral excretion has not been demonstrated for kittens of the minimal age recommended and administered the minimal recommended dose. This claim is, therefore, not accepted.

Protection against feline calicivirosis

The determination of the minimal protective dose for the calicivirus component was supported by two studies. A first study was performed in 10 week old cats administered two inactivated calicivirus antigens (FCV strains G1 and 431) with an antigen content of 2.2 ELISA units and challenged by a heterologous strain 4 weeks after vaccination. The clinical score in the group vaccinated with both antigens was lower than in the control group while not statistically significant (probably due to statistical analysis including other vaccinal groups). There was no correlation between neutralising antibody titre and protection. No clear conclusion can be drawn from that study. Another study was performed in 8-9 week old cats administered the two inactivated antigens FCV strains G1 and 431 with an antigen content of 2.0 and 2.4

ELISA units and challenged by heterologous strain 4 weeks after vaccination. Both vaccines were able to significantly reduce clinical signs and viral excretion following challenge.

More than one year after vaccination, the Purevax RCPCh FeLV vaccine with an antigen content of 2.07 ELISA units for the Calicivirus component induced a significant reduction of clinical signs and of viral excretion after a heterologous FCV challenge. However, vaccination with Purevax RCPCh FeLV vaccine with an antigen content of 2.37 ELISA units FCV induced only a significant reduction of viral excretion after challenge. Reduction of clinical signs was not statistically significant in the group receiving the highest vaccinal dose. The difference between the two vaccinated groups was, however, not significant and may be explained by the intrinsic variability of the challenge model.

Considering all the data provided, the claim of reduction of clinical signs and of viral excretion of feline calicivirosis is demonstrated.

Protection against feline panleucopenia

For the panleucopenia component, one injection of the Purevax RCPCh FeLV vaccine at a titre of $10^{3.2}$ $CCID_{50}$/dose administered to 8-9 week old cats and challenged 3 weeks later protected cats against leukopenia, clinical signs and death.

The panleucopenia component at a titre of $10^{3.2}$ CCID50/dose (0.3 log lowest than the minimum recommended) was shown to protect against feline panleucopenia 17 months after the second vaccination. A new study showed protection more than 3 years after basic vaccination using the minimal recommended dose. The SPC has been amended accordingly.

Protection against feline leukaemia

For the FeLV recombinant canarypox component, the determination of the minimum protective dose was supported by several studies. The minimal protective dose of $10^{7.2}$ CCID50 of vCP97 has been demonstrated using single or combined vaccines including this strain. For the Purevax RCPCh FeLV vaccine, administration of the vaccine containing $10^{7.6}$ CCID50/dose vCP97 to 8-9 week old cats and then challenged 2 weeks later resulted in a protection of 80% against persistent infection. The minimum titre ($10^{7.2}$ CCID50/dose vCP97) was not used for the investigated component vCP97 using the present combined vaccine Purevax RCPCh FeLV. However, considering the absence of interference with the other components, there is no reason to suspect non efficacy of the combined vaccine containing the minimal titre. The SPC claim of prevention of latent infection was not adequately supported by data.

In the duration of immunity study, none of the groups vaccinated with the Purevax RCPCh FeLV showed significant protection compared to the control adult group because of a low persistent infection rate which can be explained since the virus is expected to infect adult cats persistently to a lower extent compared to young animals. However, the duration of protection of the FeLV component alone at $10^{7.2}$ CCID50 was already proven for the EURIFEL FeLV. Considering that similar results for Eurifel FeLV and Purevax RCPCh FeLV at circa 7.5 log 10 CCID50/dose are seen, therefore absence of interference with the other components, there is no reason to suspect that the Purevax RCPCh FeLV containing the minimal titre 7.2 log 10 CCID50/dose vCP97 will not be efficacious.

Onset of protection

Based on the challenge studies the onset of protection is of 4 weeks for feline infectious rhinotracheitis and Calicivirus infection and of 2 weeks for feline panleucopenia and for feline leukaemia.

Interference with maternally derived antibodies

No interference with maternally derived antibodies is expected if vaccination is performed at 8 weeks of age against FCV, FHV-1. The recombinant canarypoxvirus cannot be neutralised by anti-FeLV antibodies. Anti-FPLV antibodies can remain at high levels at 8 weeks of age. The SPC mentions in section 5.8 that in presence of high levels of maternally derived specific antibodies, the basic vaccination scheme should be delayed until 12 weeks of age.

Field trials

Two multicentric field trials were conducted with batches of intermediate potency. In the first one, the basic vaccination schedule was applied and the second consisted of the booster vaccination being given. In both studies efficacy was based on serology, except for the recombinant FeLV component because of the absence of seroconversion. No animals of the minimum recommended age were included in the study.

The absence of field study for the leukaemia component has been justified.

The first study (basic vaccination) showed a serological response against FHV, FCV and FPV. In the second study (booster vaccination) the high antibody titres against FHV, FCV and FPV on D0 made it difficult to demonstrate the booster effect of vaccination. However, average antibody titres remained stable at a high level or increased moderately.

A major deficiency identified in the field studies was the absence of young kittens. Given the fact that the main difference between SPF and conventional kittens is the presence of MDA and considering the difficulties to recruit young kittens in field trials, a laboratory experiment was designed to induce maternally derived antibodies in SPF kittens. This laboratory trial confirmed that anti-FPV MDA is the main concern in kittens and a SPC warning has been included accordingly.

5. BENFIT-RISK ASSESSMENT

Purevax RCP FeLV is an associated vaccine containing live and inactivated components: a modified live feline herpesvirus (F2 strain), a combination of two inactivated purified caliciviruses (FCV431 and FCVG1 strains), a modified live infectious panleucopenia virus (strain PLI IV) and a recombinant canarypox-FeLV (strain vCP97) against feline leukaemia. The vaccine consists of a freeze-dried pellet containing the active ingredients of feline viral rhinotracheitis, calicivirosis and infectious panleucopenia to be reconstituted with a solvent containing the recombinant canarypox-FeLV active ingredient. The active ingredients of feline herpesvirus and caliciviruses are new strains in Europe. The feline panleucopenia virus and the recombinant canarypox-FeLV are already known, since they are components of the centralised vaccine Eurifel RCPFeLV.

The analytical part is well documented. The production and control of starting materials follows the recommendations of the EU note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01-Rev.1).

As for the part IIH, the environmental risk assessment for the genetically modified canarypox virus has been satisfactorily addressed, taking into account the remarks that were expressed during the environmental assessment of that same GMO used as a component of two previously assessed centrally authorised vaccines (The EPARs for Eurifel FeLV and Eurifel RCP FeLV can be found on the EMEA website).

The results of the overdose study in cats showed transient hyperthermia occurring 4 hours after the injection and lasting certainly 24 to 48 hours, exceptionally 5 days. Hyperthermia was still observed after 72 hours in 50% of the animals. No other systemic reactions were observed. Local reactions at injection site were transient pain and swelling. A significant decrease in the number of circulating leukocytes was frequently observed. Following the repeated administration of one dose, transient hyperthermia was rarely observed. Local reactions were transient pain and swelling. Repeated injections do not amplify the adverse effects already observed following the administration of an overdose. A significant decrease in the number of circulating leukocytes was frequently observed after the second injection.

A study was carried out in cats to evaluate local safety of the Purevax RCP FELV vaccine by histology. In all injection samples, only minimal to moderate reactions could be observed. On two occasions, no reaction could be detected at histological examination. In conclusion, the local safety of the non adjuvanted combined Purevax vaccine, administered by subcutaneous route was good and the reactions at injection site were mild to moderate. No specific study has been carried out to evaluate the impact on reproductive performance. The SPC accordingly recommends not to vaccinate pregnant animals.

The examination of immunological functions was focused on the leukopenic properties of panleucopenia and leukaemia components. Concerning specific immunity, histological examination of the thymus showed hypoplasia with a decrease in the number of cells of the cortical substance. This effect is not unexpected with a live panleucopenia vaccinal strain. No interaction study is presented. This is reflected in the SPC.

Two field trials were conducted including 115 cats of different age and breed. The general reactions observed were apathy and/or anorexia, rarely hyperthermia. In most cases they were transient (less than two days). It should be noted that lethargy and apathy observed in the field were not recorded in laboratory trials. These reactions are, however, not unexpected and are appropriately mentioned in the SPC. The local reactions were pain at injection site and swelling, as already observed in laboratory trials.

No potential risk for the environment is expected.

All the efficacy trials were carried out in the feline species, in young and adult animals. The dose used in the efficacy trials had a volume of 1 ml (claimed quantity for a dose of this product). Vaccines were administered via the recommended subcutaneous route of administration, according to the schedule of vaccination. The influence of maternally derived antibodies was satisfactorily discussed.

The efficacy of the vaccine in conventional kittens of the minimal recommended age for vaccination has not been investigated considering the difficulties to perform adequate serological and challenge studies in young conventional kittens. This was considered as justified regarding the data provided on the kinetics of maternally derived antibodies (MDAs). The main concern is FPV, for which interference with MDA is well known. Therefore the sentence "in the presence of high level of MDA, the primary vaccination course should be delayed until 12 weeks of age" has been added in the updated SPC.

Vaccination of 8 week old cats with Purevax RCP FeLV vaccine at a minimal titre of FHV provided a significant reduction of clinical signs against challenge performed 4 weeks and 13 months after vaccination. The effect on global virus excretion was however insufficiently documented and therefore the proposed claim on reduction of viral excretion was not supported.

The claim of reduction of clinical signs and of viral excretion of feline calicivirosis is demonstrated.

The panleucopenia component was shown to protect against feline panleucopenia more than 3 years after basic vaccination.

For the FeLV recombinant canarypox component, protection against persistent viraemia and clinical signs was supported by several studies.

Two multicentric field trials were conducted where efficacy was based on serology, except for the recombinant FeLV component because of the absence of seroconversion.

Serological responses against FHV, FCV and FPV have been demonstrated following basic vaccination. Following booster vaccination the high antibody titres against FHV, FCV and FPV on D0 made it difficult to demonstrate the booster effect of vaccination. However, average antibody titres remained stable at a high level or increased moderately.

A major deficiency identified in the field studies was the absence of young kittens. Given the fact that the main difference between SPF and conventional kittens is the presence of MDA and considering the difficulties to recruit young kittens in field trials, a laboratory experiment was designed to induce maternally derived antibodies in SPF kittens. This laboratory trial confirmed that anti-FPV MDA is the main concern in kittens and a SPC warning has been included accordingly.

Therefore, it could be concluded that the vaccine is efficacious when administered as described in the SPC.

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