SCIENTIFIC DISCUSSION

Invented Name:	**PUREVAX FELV**
Active substance / INN:	vCP97 recombinant canarypoxvirus (FeLV)$10^{7.5}$ CCID50 (Cell Culture Infectious Dose 50%)
Target species:	Cats
Therapeutic indication:	Active immunisation of cats of 8 weeks of age or older against feline leukaemia for the prevention of persistent viraemia and clinical manifestations of the disease.
Withdrawal period:	Not applicable
Pharmaceutical forms:	Suspension for injection
ATCvet code	QI06AD
Pharmaco-Therapeutic Group	Vaccine
Marketing Authorisation Holder	MERIAL

I. SUMMARY OF THE DOSSIER.

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

Purevax FeLV was originally authorised as a powder for suspension for injection to be reconstituted with its diluent before use. The additional pharmaceutical form of suspension for injection was subsequently granted and in 2007 the original presentation of powder with suspension for injection was deleted.

QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE CONSTITUENTS

COMPOSITION OF THE VETERINARY MEDICINAL PRODUCT

Active substance:
Each 1-ml dose of vaccine contains:
vCP97 recombinant canarypoxvirus (FeLV)$10^{7.5}$ CCID50 (Cell Culture Infectious Dose 50%)

Other ingredients:

Excipients:

Gentamicin sulphate (trace)
Lactose
Glutamic acid
Monopotassium phosphateDipotassium phosphatePotassium hydroxide,
Sodium chloride,
Disodium phosphate dihydrate,
Monopotassium phosphate,

Constituents of the diluent (Ph. Eur.):

Water for injection, 1 ml

Adjuvants: None

Preservatives: None

CONTAINER

The container is either a Type I glass colourless bottle (Ph.Eur.) with a butyl elastomer closure (Ph.Eur.) and an aluminium cap or a Type I glass syringe with a plunger-stopper and an elastomer needle-cover.

CLINICAL TRIAL FORMULATIONS

- The vaccine dose administered to the cats included in the different trials always complied with the vaccination schedule recommended in the instructions for use. Its active ingredient content was at least equal to the maximum titre claimed by the manufacturer for a volume of 1 ml, i.e. at least 108.4 CCID50, except in some trials

- The samples used for the trials were always taken from vaccine batches produced in accordance with the manufacturing process described by the manufacturer in the analytical dossier of application for marketing authorisation. However, for some trials, a supplementary treatment stage was added to the classical manufacturing process.

- During some trials, the following vaccine components were combined to Purevax FeLV vaccine or used in parallel to the Purevax FeLV vaccination: attenuated live vaccine against Feline Infectious Panleukopenia; inactivated vaccine against Feline Viral Rhinotracheitis and Calicivirosis.

PRODUCT DEVELOPMENT STUDIES

Choice of the strain:

The use of a strain from subgroup A (FeLV-A), as donor strain for the inserted genes is justified by the claim that FeLV-A is always present in natural isolates of FeLV and FeLV-B or FeLV-C groups are only occasionally present, horizontal transmission of subgroups B & C requires infection with strains of group A, that there is strong gp-70 cross-reactivity of neutralising antibodies between isolates and by the published observation that a vaccine containing a strain of subgroup A conferred protection against a challenge containing a blend of the three subgroups.

The inclusion of genes encoding for env, gag and part of pol is justified by the desire to obtain an immune response against a large range of antigens, including not only neutralising antibodies against gp70 but also cytotoxic T lymphocytes against the other structural proteins.

The choice of the live virus vector system is not discussed in the quality expert report but it is in the dossier. The use of a live virus vector system is motivated by the experience of the company with such vectors, the fact that canarypox virus does not multiply in the host (cat) but expresses the antigen in-vivo and that no adjuvant is needed for such vaccines.

Choice of the antigen concentration:

A dose-response relation has been determined in a vaccination/challenge study. The minimum protective titre dose of $10^{7.3}$ $CCID_{50}$ was established as the dose able to protect at least 80% of the kittens.
The maximum release titre was set at $10^{8.4}$ $CCID_{50}$ (1/10 of the maximum dose with demonstrated safety).
The minimum release dose is equal to the minimum protective dose, since no stability loss could be detected after storage at +5°C±3°C during the claimed shelf-life of the product. The minimum protective dose was fixed at 7.5 log10 CCID50 per dose and validated in an immediate efficacy study in compliance with the European monograph (part IV) and in a duration of immunity study (part IV). It must nevertheless be emphasized that the minimum protective dose is in fact less than 7.5 log10 CCID50 per dose; the dose-response study (part IV) demonstrated that the minimum calculated dose of recombinant virus to protect 80% of the cats, thereby fulfilling the criteria of European Pharmacopoeia, was 7.24 log10 CCID50 per dose. A safety margin of 0.26 log10 CCID50 is therefore present.

Potency of the vaccine is assessed by titration of the canarypoxvirus on cells. This technique was thoroughly validated

On 12 May 2003 the European Commission approved a Type II variation to reduce the minimum titre of the vaccine from $10^{7.5}$ to $10^{7.2}$ CCID50 (cell culture infective dose 50%).

Choice of the adjuvant:

No adjuvant is added in the formulation.

Preservative:

No preservative is added in the formulation.

Other excipients

The choice of the excipient and diluent was adequately documented. The excipients lactose, glutamic acid, monopotassium phosphate, dipotassium phosphate, potassium hydroxide were used as virus freeze-drying stabilisers; the diluent and the components of the buffer: sodium chloride, disodium phosphate dihydrate, and monopotassium phosphate were used for the reconstitution of freeze-dried vaccine and volume adjustment buffer for formulation.

Filling Volume

The volumes of virus suspension and excipient solution/buffer diluent are calculated from the infective titre on cells.

Choice of the potency testing technique for the finished product:

A trial was designed to establish the efficacy of the canarypox-FeLV vaccine administered in the dose previously found in the dose response trials to be required for significant protection against oronasal FeLV challenge. The vaccine contained the canarypox-FeLV recombinant virus at a concentration adjusted to $10^{7.5}$ $CCID_{50}$ in the inoculated dose.

Definition of the norms:

A dose response study was undertaken to determine the minimum dose of the recombinant virus required for protection against an appropriate FeLV challenge. A series of three trials was conducted under similar conditions.
Specific pathogen free kittens of 8-10 weeks of age were vaccinated on two occasions, 3 weeks apart with various doses of vCP97 canarypox recombinant virus and then challenged 2 weeks after the second dose of vaccine with FeLV-A given by the oronasal route to simulate natural infection.
The kittens were then monitored at intervals of three weeks for 12 weeks to determine their virus status.
In the control groups, the challenge virus induced persistent infection in 83%.
The minimum calculated dose of recombinant virus to protect 80% of cats, thereby fulfilling the criteria of the European Pharmacopoeia, was $10^{7.24}$ $CCID_{50}$.

QUALITATIVE AND QUANTITATIVE PARTICULARS OF THE SUSPENSION FOR INJECTION

The starting materials used in the production of the liquid vaccine remain unchanged in relation with the freeze-dried vaccine, except for calcium chloride dihydrate and magnesium chloride

hexahydrate that are included in the proposed liquid formulation. These salts are compliant with their respective Ph.Eur. monographs 0015 and 0402.

The change of pharmaceutical form induced a change in the composition since PBS is used instead of the freeze-drying stabiliser and buffered physiological saline used for the formulation of the powder.

The limits of acceptance of the vCP97 components in the liquid vaccine remain unchanged and are as follows:

	vCP97 AI
Minimum protective dose	$10^{7.2}$ $CCID_{50}$/dose
Minimum release dose	$10^{7.5}$ $CCID_{50}$/dose
Target titre	$10^{8.1}$ $CCID_{50}$/dose
Maximum release dose	$10^{8.4}$ $CCID_{50}$/dose

The dose volume of the liquid presentation is identical to that of the reconstituted freeze-dried presentation. In order to guarantee that the injected volume corresponds to at least the dose volume (1 ml), a volume of 1.05 ± 0.005 ml is filled in each bottle of vaccine.

Since the compositions of the two pharmaceutical forms were identical in their viral titre, no impact is foreseen on the efficacy and the safety of the vaccine. In particular, the BSE/TSE risk assessment remains unchanged. The lactose included in the freeze-dried presentation is no longer present in the liquid form. This is in line with the Ph. Eur. monograph 0062 which recommends to reduce, wherever practicable, the use of substances of animal origin.

DESCRIPTION OF METHOD OF PREPARATION OF THE FINISHED PRODUCT

FORMULA AND PROCESS

The volumes of virus suspension and excipient solution/buffer diluent are calculated from the infective titre. This bulk is stored before filling, freezing, freeze-drying and capping. Examples of three batches were presented.

VALIDATION STUDIES

The control tests on the MSV (Merial Master Seed Virus) are in compliance with General Requirements for the Production and control of Live Mammalian Bacterial and Viral Vaccines for Veterinary Use (III/3182/91 and Ph. Eur. 6.2.). The techniques different from those recommended by the Ph. Eur. were validated.

METHOD OF PREPARATION OF THE SUSPENSION FOR INJECTION

1. Formulation

The target titre was set at $10^{8.1}$ $CCID_{50}$. The appropriate volume of virus active ingredient is determined from its concentration before freezing. The necessary volume of PBS is added in order to obtain the desired volume (1 ml). The PBS solution is prepared with water for injection by weighting, mixing and homogenisation of the different constituents (potassium chloride, sodium chloride hexahydrate and calcium chloride dihydrate).

The buffer is sterilised by filtration through a 0.22 µm filter. The feasibility of PBS heat sterilisation has been addressed through a study involving three batches (Ref 04.0231.R). Heat

sterilization appears to slightly modify the pH and osmolarity. However, the modification is enough to make that pH values fall out of the limits of specification. Moreover, the appearance of the three batches is significantly modified with formation of a significant precipitate. For these reasons, filter sterilisation has been preferred. The bulk is homogenised and stored at +5°C (±3°C) until filling for at most 2 months.

The size of the blend can be included between 40 and 400 litres and the batch size between 40,000 and 400,000 doses.

2. Filling and packaging

The filling is carried out in a clean area under laminar flow of grade A. The bulk is aliquoted in washed and dry heat sterilised bottles through steam or gamma-radiated connecting pipes or transferring vessels. Closure are washed, silicon-coated and steam sterilised. Closed bottles are then crimped with a cap and gathered in boxes until secondary packaging.

3. Finished product

After printing the batch number and the expiry date, labels are stuck on containers and the latter are introduced in boxes, together with the package insert. Quality controls are performed at each step of the secondary packaging operation. Finished products are stored in cold room at +5°C (±3°C) until dispatching.

An example of the preparation of 3 batches was described and an updated manufacturer's batch protocol was provided detailing the active ingredient titre.

During the developmental phase of the liquid presentation of the vaccine, a target formulation titre of $10^{7.9}$ CCID50/ml was tested with the objective to decrease the formulation target. However, as the specification range remained narrow and minor stability losses were observed, the formulation target was finally kept at $10^{8.1}$ CCID50/ml to allow for more flexibility at re-testing time and compensate for those losses.

It was shown that higher release titres were obtained from batches formulated with a target titre of $10^{8.1}$ CCID50/ml. The target formulation titre of $10^{8.1}$ CCID50/ml should therefore guarantee the minimal titre throughout the proposed shelf-life.

The bulk can be stored between +2°C and +8°C until filling, for at most 2 months. The only further manufacturing step is the filling: the bulk stored in vessels remains therefore physically identical to the product filled in vials (liquid vaccine, same storage temperature).

The vaccine batches used in the stability study were tested for potency (by infectious titration test) almost one month after their blending/filling date. Therefore, a one-month storage period before T0 has been taken into account in the stability study.

CONTROL OF STARTING MATERIALS

STARTING MATERIALS LISTED IN PHARMACOPOEIA

Glutamic acid
Sodium hydrogen carbonate Sodium chloride
Disodium phosphate dihydrate
Potassium chloride
Potassium dihydrogen phosphate
Sodium dihydrogen phosphate dihydrate
Trisaminomethane
Water for injection (in bulk)
Purified Water
Potassium hydroxide
Lactose monohydrate
SPF embryonated hen eggs
Dipotassium phosphate
Gentamicin sulphate
Glass containers for pharmaceutical use (vial)
Butyl elastomer closure
Butyl elastomer plunger stopper
Glass containers for pharmaceutical use (syringe)

The certificates of analysis of these starting materials have systematically been supplied. Wherever necessary, the certificates specify the quantitative results of the control tests.

STARTING MATERIALS NOT IN A PHARMACOPOEIA

Starting materials of biological origin

1. SPF chick embryo cells
2. Vaccine virus strain
3. Calf serum
4. Foetal calf serum
5. Pronase
6. Tryptose phosphate broth

SPF chick embryo cells:

These primary cells, used for the production of the active ingredient, are isolated from SPF hen eggs, raised and tested in accordance with current requirements.

Vcp97 vaccine virus strain

Origin:
The strain was obtained by recombination between the canarypoxvirus Alvac strain and sequences isolated from the Glasgow 1 strain of the feline leukaemia virus subgroup A (cDNA sequences). The canarypoxvirus strain was attenuated in order to obtain the vaccine strain used in the Kanapox® vaccine. This vaccine strain was then amplified to obtain the Alvac vector.

Construction of the virus:
The constructions are described.

First step: Genetic engineering

The env gene, under the control of the promoter H6, was inserted by a first recombination. The resulting virus was cloned seven times by the method of plaques, with satisfactory homogeneity of the clone.

The gag gene + 1272 bp 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 the clone called vCP97. The arguments supplied concerning the purity of the clone are satisfactory. Data on the boundaries of the inserted sequences are detailed.

Second step: Purification

The vCP97 clone has again been purified by the method of plaques.
The conditions of recombination and cloning seem to ensure the satisfactory homogeneity of the MSV, the conformity of the construction of the vCP97 clone was verified

VERIFICATION OF THE EXPRESSION OF INSERTED SEQUENCES:

Expression of env and gag/pol genes was fully demonstrated using immunological methods
This expression was confirmed in the genetic stability study

STABILITY OF THE CONSTRUCTION:

The stability of the construction was verified through (5 passages , MSV+5).

Controls on the Master Seed Virus

The control test on MSV is in compliance with General Requirements for the Production and control of Live Mammalian Bacterial and Viral Vaccines for Veterinary Use (III/3182/91 and Ph. Eur. 6.2).

Controls on the Working Seed Virus:

The Working Seed virus (WSV) was subjected to classical bacterial, fungal and mycoplasmic sterility tests (in compliance with the Ph. Eur.) as well as to an identity test and titration.

Calf serum and foetal calf serum

Tests comply with the recommendations of guideline EEC III/3182/91-EN (live vaccines).
A declaration has been provided that the Applicant is compliant with the requirements set out in the EU guidelines and in Commission Decision 97/534/EC.

Pronase

Viral, bacterial, fungal and mycoplasmic sterility are adequately guaranteed.

Tryptose-phosphate extract

Viral bacterial, fungal and mycoplasmic sterility are adequately guaranteed.

Starting material of non-biological origin

These are: buffered physiological saline, Freeze-drying substrate, Tris buffer pH 9, Lactoglutamate buffer, PBS), Hydochloric acid and Cell culture medium. Specifications are presented in the form of monographs. The assay techniques correspond generally to techniques described in the European Pharmacopoeia, or are sufficiently described. The certificates of analysis of the starting materials have been systematically supplied.

STARTING MATERIALS FOR THE SUSPENSION FOR INJECTION

Listed in a Pharmacopoeia

The starting material used in the production of the liquid vaccine remain unchanged in relation with the freeze-dried vaccine, except for calcium chloride dihydrate and magnesium chloride hexahydrate which are used in the preparation of the PBS for the formulation of liquid vaccine.

Calcium chloride dihydrate and magnesium chloride hexahydrate respectively comply with the Ph. Eur. monographs 0015 and 0402 and certificates of analysis were provided.

Not listed in a Pharmacopoeia

The starting material of biological origin, most of the starting materials of non-biological origin and the in-house prepared media remain unchanged. A description of the PBS preparation and its testing (pH, osmolality, sterility) as well as a certificate of analysis was provided.

CONTROL TESTS CARRIED OUT AT INTERMEDIATE STAGES OF THE MANUFACTURING PROCESS

1. In-process controls during formulation, filling and packaging

The tests carried out examine formulation, filling, freeze-drying, packaging, the diluent and the secondary packaging. Results of in-process testing were provided for three production batches and the in-process controls were found to be adequate.

2. In-process controls on the active ingredient

These are discussed as routine tests on the active ingredient (II.C.2.1.). The active ingredient was tested for bacterial and fungal sterility, as well as for infectious titre.

CONTROL TESTS DURING PRODUCTION OF THE SUSPENSION FOR INJECTION

Control tests are carried out on the formulation, at filling, for primary packaging and for secondary packaging

The batches presented in the dossier were representative pilot batches only. The batches were produced in small volumes, and in consequence industrial standard operating procedures for in-process controls were not fully applied.

The in-process controls during formulation, filling and packaging were themselves considered adequate. The Marketing Authorisation Holder has committed to providing the results of in-process testing for at least three production batches.

CONTROL TESTS OF THE FINISHED PRODUCT (LYOPHILISATE)

The complete list of tests of the finished product is presented below:

Test on Lyophilisate
Appearance
pH
Identity
Live virus concentration
Specific safety
Bacterial and fungal sterility
Mycoplasma
In-vitro extraneous agents
Residual moisture
Appearance
Extractable volume
Acidity
Alkalinity
Oxidisable substances
Chlorides
Nitrates
Sulphates
Ammonium
Ca & Mg
Heavy metals
Residue on evaporation
Endotoxins

The certificates of analysis of three small pilot batches of lyophilisate and six batches of diluent (three bottles and three syringes) have been supplied. The results presented in this section are compatible with adequate production consistency.

CONTROL TESTS ON THE FINISHED PRODUCT (SUSPENSION FOR INJECTION)

The following tests were carried out.

Appearance, pH, volume, identification of vCP97 AI, titration of vCP97 AI, safety test, bacterial and fungal sterility, mycoplasmic sterility and viral purity.

Control tests (technique and limits of acceptance) performed on the finished product remain unchanged except for those in relation with the general characteristic and the residual humidity.

The batch-to-batch consistency has been addressed through the analysis of three batches, which display compliance with the specifications. Their corresponding certificates of analyses have been provided.

Some local reactions were induced however these were in line with those observed in the safety studies conducted for the freeze-dried product. An appropriate statement referring to temporary small nodules has been included in the Summary of the Product Characteristics.

STABILITY

Stability of the bulk

Data have been provided to show that the bulk is stable.

Stability of the finished product

The proposed shelf-life of the finished product is 12 months at 2-8°C. The stability study has been carried out on three pilot batches of lyophilisate and six batches of diluent (three in bottles and three in syringes).
The Applicant has committed to provide further stability data on the first 3 production-scale batches and stability data at real time and temperature a soon as these become available.

Stability of the reconstituted product

The results of batches of lyophilisate reconstituted with diluent have been supplied.

In-use shelf-life

The product is to be used immediately after reconstitution.

STABILITY OF THE SUSPENSION FOR INJECTION

A stability study was carried out on three batches of finished products. The physico-chemical parameters (volume, appearance and pH) remain unchanged during the storage period. The three batches were sterile at T0 and after 15 months of storage. However, one of the batches appeared contaminated at T27 months.

Titration of these three batches were conducted all over the stability study period. After 27 months, the average loss of titre is –0.29 $\log_{10}CCID_{50}$/ml, justifying the margin between minimum protective dose and minimum release dose (0.3 $\log_{10}CCID_{50}$/ml). Among the three batches, only one gave fully satisfactory results.

For the vCP97 (canarypox-FeLV) component, release specifications are quite narrow and make it more likely to find a titre out of specification. This risk is inherent to the formulation of this product. This risk was increased in the stability study due to the repeated nature of the test. The stability batches were formulated according to a target titre of $10^{7.9}$ CCID50/ml. Due to the slight loss observed in stability, formulation target was set at $10^{8.1}$ CCID50/ml. This change will guarantee the minimal titre throughout the proposed shelf-life.

The stability of suspension for injection was confirmed by performing a re-titration of batches manufactured up to 22 months ago to estimate the titre loss by comparison to the titre at T0.

All vaccines tested at T0 had titres above the minimum release titre (i.e. $10^{7.5}$ CCID50/dose), and all vaccines tested between 14 and 22 months of shelf-life had titres above minimum protective dose (i.e. $10^{7.2}$ CCID50/dose). Notwithstanding the age of the batches, the average titre loss was 0.25 log10 confirming thereby the model established for the liquid Purevax FeLV vaccine.

The Marketing Authorisation Holder made the commitment to place the first 3 manufacturing scale batches on long-term stability after approval.

The Batch protocols of the three batches used in the stability study: 0 RM678 5D041, 0 RM678 5E051 and 0 RM678 5F061 were provided.

Whilst the mycoplasmic and viral purity control tests were not performed on these stability batches, the absence of mycoplasmic and viral contamination was demonstrated through the results obtained for other vaccine batches (i.e. designed "representative vaccine batch for control test") produced with the same active ingredients as for the stability batches. The results were considered satisfactory. It was confirmed that mycoplasmic and viral purity control tests will be performed on a routine basis on a final lot of each batch.

No data was provided regarding stability of the vaccine during un-chilled transport as the recommendation is to maintain the product at 2 °C-8°C until use and an appropriate text appears under point 6.3 (Special precautions for storage) of the SPC ("Store and transport at 2°C – 8°C").

ENVIRONMENTAL RISK ASSESSMENT FOR PRODUCTS CONTAINING GENETICALLY MODIFIED ORGANISMS.

In order to evaluate the risk related to GMOs the interaction between the GMO and the environment must be studied. In particular, the risks of spread from the vaccinated animal must be analysed by studying 1) the likelihood of spread to other organisms either directly or indirectly depending on available channels 2) the consequences of a possible spread 3) how to manage these risks.

The dossier II.H submitted analyses these different issues by following the format suggested by the corresponding guideline. The reports are, for most of them, also included in the dossier as part of the pharmaceutical quality, safety and efficacy data and have thus been evaluated in the corresponding expert reports. In particular, the construction of vCP97 as well as its stability have been analysed in this report.

As recommended in the guideline, all the possible theoretical risks were considered and the likelihood of hazard was analysed as well as the consequence of hazard, in order to determine each level of risk.

The foundation of the risk analysis for this type of product is the fact that the ALVAC strain does not multiply in the target species (cats) and, more generally, in mammals. It is obvious that this is an element of biosafety princeps, on which all the other parts of the analysis are based. Therefore, if the data submitted demonstrate this feature adequately, all the other data in the dossier would only constitute a complementary demonstration. The data supplied to demonstrate this feature were analysed and the other data supplied as a confirmation of the risk analysis were also considered.

The liquid formulation is not expected to have any impact on the environmental risk assessment of the canarypox GMO.

RESTRICTION OF THE REPLICATION OF CANARYPOXVIRUS (ALVAC VECTOR) IN MAMMALS

Restriction of replication in cell culture

The reports demonstrate convincingly that Alvac does not replicate in any of the mammalian cell lines tested.

Restriction of replication in the animal

Report evaluates the viral excretion from cats, after subcutaneous administration at a high dose. It demonstrates the absence of virus isolation from saliva, urine and faeces samples. Additional information may be used as indirect information: they analysed the pathogenicity of the virus under different severe experimental conditions, or else, they analysed the persistence of the virus beyond the inoculation site.

Analysis of the risk to escape host range restriction

Analysis of the risks and the consequences of possible complementation

Since the defective genes at the origin of the attenuation of the strain are not known, it is difficult to estimate theoretically the risks of complementation, either by cellular or viral genes.

As far as a cellular complementation is concerned, the data obtained in the cat are nevertheless an indication of the absence of this type of mechanism at a sufficient level in the target cells after a subcutaneous injection to obtain excretion. The risks of complementation by other viruses infecting the same cells must also be analysed. This type of in vivo risk can be analysed as follows:

a) First, it is necessary to have co-localisation of the two viruses,

b) Second, it is necessary to have infection of the same cells by both viruses.

c) Third, it would be necessary to have complementation at the molecular level.

d) Finally, for such a mechanism to have an epidemiological significance, a balance should exist between the replication of both viruses.

The consequences of such a risk seem minimal. Indeed, no adverse effects on the health of cats or cats in contact with the virus, expressing the gag, env genes and part of the pol gene inserted in the vector, are expected.

Analysis of the risks and consequences of possible recombination

Recombination risks mainly concern the insertion of sequences of viral origin in the vCP97 which could cause spread. Again, for phylogenic proximity reasons, the main issue to be analysed is a possible recombination with the cowpox virus.

a) The first necessary steps are those described in parts a and b of paragraph 1.3.1.

b) idem

c) the risk of recombination depends on the homology between sequences.

The cumulated risk for such events is thus very low.

Analysis of the risks of mobilisation of the retroviral genes inserted in the vector

Two hypotheses must be taken into account: recombination that would allow the insertion of FeLV sequences inside the genome of a virus superinfecting the cells transducted by vCP97; and mobilisation in FeLV pseudoparticles of FeLV sequences inserted in vCP97.

Insertion of FeLV sequences by recombination in the genome of an exogenous virus

For the same taxonomic proximity reasons mentioned above, the risks of recombination concern mainly an insertion of FeLV sequences in the genome of a cowpox virus.

a) The first necessary events are those described in parts a and b of paragraph 1.3.1.

b) idem

c) In case of co-localisation of the genomes inside the same cell, the risk of recombination depends on the homology between sequences.

Thus we do not consider there is any specific risk associated to this type of recombinant.

Involvement of FeLV sequences inserted in vCP97 in FeLV pseudoparticles

The capacity of vCP97 to produce pseudoparticles after infection has been documented. No image of pseudoparticle has been evidenced.

OVERVIEW

In all the hypotheses, the analysis demonstrates a very low likelihood of hazard and very insignificant clinical and epidemiological consequences. Consequently, the risk of loss of replication restriction in cats seems close to zero.

OTHER DATA SUPPLIED FOR THE ANALYSIS OF RISK

The manufacturer has supplied additional arguments for the analysis:

The single-dose presentation of the product and the injection administered by a veterinarian reduce the likelihood of accidental spread, additional data show the very limited risk of spread into the environment, as well as the absence of known risk of amplification in mammals due to the defective vector and, more specifically, the absence of risk in humans.

CONCLUSION

The data supplied by the manufacturer for a risk analysis and complemented by available scientific data are mainly based on the defective character of the vaccine strain for replication in mammals, in particular in cats and humans.

Further to an oral explanation provided by the applicant, it is considered that Purevax FeLV does not show any documented risk. Moreover, based on current scientific knowledge and virological concepts, we do not identify any foreseeable risk associated to its use under the conditions described. Furthermore, the data presented by the company in its dossier are in accordance with the Scientific Advice provided by the CVMP.

OVERALL CONCLUSIONS ON QUALITY

The quality of the product was found to be acceptable. The Marketing Authorisation Holder has committed to provide the results from the first 3 manufacturing scale batches being placed on long-term stability and the concomitant in-process control results.

III OVERVIEW OF PART III OF THE DOSSIER: SAFETY

INTRODUCTION

The different safety studies were carried out in compliance with the requirements of Directive 81/852/EEC as amended (now Directive 2001/82/EC).

GENERAL REQUIREMENTS

The safety trials were carried out in the target species, i.e. cats, on both young and adult animals. Some of the trials were carried out on other species (canaries, mice, guinea pigs) in order to provide evidence of the vaccine safety in these species.

The vaccine used was that described in the Summary of Product Characteristics (SPC) included in the dossier. The vaccine dose administered to the cats included in the different trials always complied with the vaccination schedule recommended in the instructions for use. Its active ingredient content was at least equal to the maximum titre claimed by the manufacturer for a volume of 1ml, except in few trials. In the Field trials in France and Belgium the vaccine used came from batches whose titre was lower than the maximum titre claimed by the manufacturer.

The samples used for the safety trials were always taken from vaccine batches (or once active ingredient) produced in accordance with the manufacturing process described by the manufacturer in the analytical dossier of application for marketing authorisation. However, for some trials, a supplementary treatment stage was added to the classical manufacturing process.

During some trials, the following vaccine components were combined to Purevax FeLV vaccine or used in parallel to the Purevax FeLV vaccination:
Attenuated live vaccine against Feline Infectious Panleukopenia;
Inactivated vaccine against Feline Viral Rhinotracheitis and Calicivirosis;
Inactivated vaccine against Feline Chlamydiosis;
Inactivated vaccine against Rabies.

In some trials, vaccines containing the same vaccine strain as Purevax FeLV but combining other vaccine components were used. Finally, Feline Leukaemia vaccines containing a different FeLV component from the vCP97 component were used during some of the trials: Competitor Product A and Competitor Product B.

All the laboratory studies have been carried out in accordance with the Good Laboratory and Clinical Practice Principles.

LABORATORY STUDIES

The evaluation of the safety of the administration of one dose is based on two trials, one carried out on specified pathogen-free (SPF) kittens at the minimum age recommended for vaccination and the other carried out on older cats infected with feline leukaemia virus (FeLV)

The studies on 8-weeks-old kittens and 7-months-old cats have been properly conducted. No information is provided on the macroscopic and microscopic lesions at the injection site. This can be accepted for non food producing animals. No study has been performed in pregnant animals, but the vaccine is contra-indicated in such category of animals. More details on the undesirable effects are included in the SPC.

The demonstration of the safety of the administration of an overdose is supported by two trials:

A total of 47 kittens were subcutaneously administered with an overdose of Purevax FeLV vaccine or active ingredients used alone. The trials were carried out on the most susceptible animals i.e. young kittens.

These trials provide evidence of the absence of abnormal general reactions, except for transient signs of apathy 4 to 16 hours following the injection of vaccine and transient hyperthermia also 4-16 hours following injection, or the next day or day after next. Local signs may also develop following the vaccine injections, mainly in the form of a limited and transient oedema. These reactions are consistent with the possible adverse reactions such as specified in the Summary of Product Characteristics (Part I.B, § 5.4) and the process including clarification does improve the safety of the vaccine.

The safety of the repeated administration of one dose (containing between 1 and 6 maximal doses) was demonstrated in two trials. The conditions in which the trials were carried out complied with the requirements of Directive 81/852/EEC and European Pharmacopoeia monograph Feline Leukaemia vaccine (inactivated) in terms of safety of the repeated administration of one dose. A total of 30 kittens were repeatedly injected.

In conclusion, both these trials demonstrate the absence of abnormal general reactions besides moderate and transient, occasional hyperthermia which may occur 4 to 8 hours following the injection of vaccine, if not the next day or day after next, whether combined with slight apathy or not. Local reactions may also appear following the vaccine injections, mainly in the form of a limited and transient oedema at the site of injection. These reactions are consistent with the possible, expected adverse reactions specified in the Summary of Product Characteristics.

EXAMINATION OF REPRODUCTIVE PERFORMANCE

No specific study was carried out in pregnant females. Therefore, the harmful effects upon the progeny and the possible, teratogenic and abortive effects are not documented. Even if, it has been demonstrated that the recombinant vaccine strain vCP97 cannot replicate and disseminate in the body of the vaccinated animal, in the absence of such data, Purevax FeLV vaccine is contra-indicated in pregnant females.

EXAMINATION OF IMMUNOLOGICAL FUNCTIONS

LEUKOPENIC REACTIONS

Vaccination does not induce any significant decrease in the number of circulating leukocytes.

SPECIFIC IMMUNITY

The interaction of Purevax FeLV vaccine with other vaccine components (attenuated live vaccine against feline infectious panleukopenia, inactivated vaccine against feline viral rhinotracheitis and calicivirosis, and inactivated vaccine against feline chlamydiosis) was evaluated and is addressed below. This trial shows that the humoral immune response against these components remains unchanged, whether Purevax FeLV is combined with other components or not.

All the above-discussed data demonstrate that vaccination does not interfere with the development of the immunological functions of cats.

Special requirements for live vaccines:

Spread of the vaccine strain

The manufacturer's rationale concerning this part of the dossier has been supported.
Eight-week old cats were subcutaneously inoculated with a high dose of FeLV (vCP97) recombinant canarypox. The safety and the spread of this recombinant virus was compared to those of the CPpp parenteral canarypox virus and those of a contact placebo control group.

Concerning the spread of the vaccine strain, the original results given in this trial clearly shows that neither the vCP97 vaccine virus nor the CPpp parental virus can be isolated from the different body secretions or excretions (saliva, urine, faeces) for 7 days following vaccination at high titres of kittens and, therefore, that the spread of the vaccine strain in the body of the vaccinated animal is very restricted.

No spread of virus from vaccinated cats is possible. Nevertheless, as recommended by Directive 92/18/EEC, it may be necessary to evaluate the spread of the vaccine strain to other species to which the vaccine is not intended but which may prove to be susceptible to a live vaccine.

Dissemination in the vaccinated animal

The trial showed that it is not possible to isolate the vCP97 vaccine virus from the excretions or secretions following vaccination.

Reversion to virulence of attenuated vaccines

This section of the dossier refers to a document appended to the safety dossier (Document EMEA/CVMP/259/96-FINAL). Given the non-replicative properties of the recombinant virus, (see below), reversion to virulence cannot take place.

Biological properties of the vaccine strain

Replicative properties

The replicative properties of the vCP97 recombinant virus were evaluated in vitro and compared to the CPpp parental strain

The results obtained show that, as opposed to cells of avian origin, the cells of mammalian origins are not permissive to the canarypox virus

SAFETY FOR OTHER ANIMAL SPECIES

Safety for canaries

This trial demonstrates the general safety of the poxvirus inoculated by the transcutaneous route to the canary despite its capacity to mainly induce cutaneous lesions.

Safety for guinea pigs and mice

This trial was the evaluation of the safety of the vCP97 vaccine virus in guinea pigs and mice following intraperitoneal (guinea pigs) or subcutaneous (mice) administration, by comparing it with the parental canarypox virus strain. No mortality, local lesion and general reaction were observed

Recombination or genomic re-assortment of strains

Report presents a study whose aim was to evaluate the possibilities of in vivo recombination between a field canarypox virus strain and a recombinant canarypox virus during a combined experimental infection in canaries.

This trial shows that there was no recombination of the recombinant virus with a field strain leading to a persistent strain in such a manner as to immortalise the gene introduced.

Regarding the recombinant status of the vaccine, both French and Belgian competent authorities authorised the deliberate release of the ALVAC-FeLV in the context of controlled open field trials. These authorisations were granted by the “Commission du Génie Biomoléculaire” in France and by the Ministry in charge of Public Health in Belgium. A copy of the Summary Notification Information Formats (referred to in art. 9 of Directive 90/220/EEC) in accordance with Directive 91/596/EEC (last modified by Directive 94/211/EEC) was presented.

An additional study was carried out to confirm the safety and non-diffusion of the ALVAC-FeLV in chickens. This study demonstrated the good local and general tolerance, and the absence of spread of the vCP97 in chickens.

In conclusion, the risk of disease in commercial poultry is nil.

Study of residues

Not applicable.

Interactions

Trial report demonstrates the safety of Purevax FeLV vaccine in relation to the use of other vaccine components.
The combination of Purevax FeLV vaccine with other vaccine components habitually used in cat vaccination protocols (attenuated vaccine against feline infectious panleukopenia, inactivated vaccine against feline viral rhinotracheitis and calicivirosis, and inactivated vaccine against feline chlamydiosis) does not have any influence upon the humoral immune response (antibody titre) against these components following vaccination.

FIELD STUDIES

Four field studies, including more than 948 cats, are included in the safety dossier in support of the laboratory tests.

The conditions in which the four field studies presented were designed and carried out were considered satisfactory as :

- These trials were carried out on a large number of cats i.e. 948 cats in total, of which 660 were vaccinated with combined vCP97 vaccine.
- These trials included cats of different breeds and sizes, particularly cats of non European breeds (called " exotic " breeds)
- These trials included cats of different ages;
- These trials were carried out following information and informed consent of the cat owners;
- All vaccinated cats in trials were carefully examined (general examination, rectal temperature recording and observation for at least 4 hours post-vaccination and daily monitoring for 14 days post-vaccination) for any sign of local or general reaction;
- The follow-up procedures of last trials were less strict due to the conditions in which they were carried out (at veterinary practices and on cats belonging to private owners) and to the large number of cats involved.

Some trials were carried out with vaccines coming from batches whose titre was lower than the maximum titre claimed by the manufacturer for a volume of 1 ml, but were representative of future commercialised batches. As required by Directive 92/18/EEC, the field trials provide additional information and confirm the data obtained in the laboratory even if carried out with a combined vaccine and not the monovalent one. The adverse reactions observed were mentioned in the Summary of Product Characteristics. These trials provided evidence of the following:

- safety of vaccine administered as a single dose or repeated doses on a large number of animals,
- safety of vaccination on both young and adult cats,
- safety in real conditions of use,
- similarity of the adverse reactions obtained following the administration of Purevax FeLV and those obtained with competitor commercial vaccines which have already been granted a marketing authorisation.

ECOTOXICITY

PRESENTATION OF THE RATIONALE

The supportive arguments given in the dossier were based upon the following:

- a summary document (bibliographical and experimental data);
- bibliographical data
- a number of trials, which have been previously analysed under section C.6;
- other trials presented

With the elements given above, safety is rigorously demonstrated including two main parts: the first part dedicated to the assessment of risk to human health and the second more generally dedicated to the assessment of the risk to the environment. This presentation results in the calculation of a very low degree of likelihood (close to zero hazard).

EVALUATION OF THE RATIONALE

Concerning the assessment of the possible degree of exposure of the product, its active ingredients or metabolites to the environment the following points make product exposure to the environment quite unlikely.

1. the target species (cats) and its method of use (individual vaccination);
2. its method of administration (subcutaneous route);
3. its presentation (single-dose containers);
4. the restriction of the replication and non-excretion of the vaccine virus from vaccinated cats;
5. and, finally, the disposal of wastes and destruction of unused vaccine contents by the veterinary practitioner.

However, since this product deals with a genetically-modified organism (GMO), the risks of a lessening in the replicative restriction should be investigated as well as the risks of mobilisation of the retroviral genes inserted in the vector. These aspects have been assessed in Part II.H of the analytical dossier

CONCLUSIONS ON SAFETY

Besides the absence of safety problems (excluding local reaction) during vaccination with poxvirus recombinants in the animal, the rationale presented is based upon the deficient replication properties of the vaccine strain in mammals, especially in cat. Moreover, considering the quite unlikely contamination of susceptible non-mammalian species, especially canaries, the lowest capacity of replication of the vaccine strain in the canary in relation to the parental strain and to the absence of documented incidents in this species during the use of Kanapox ™ vaccine, support the safety of Purevax FeLV vaccine in this case.

As a conclusion, and in the limit of the conclusions of Part II.H, the documents presented in the safety dossier (Part III) are satisfactory and rule out, in the present state of our knowledge, the possible ecotoxicity of the vaccine. However, the recommended destruction of unused vaccine contents remains a wise precaution.

The safety dossier includes 21 trial reports, supported by bibliographical data. The trials supplied by the manufacturer are acceptable and give all the necessary guarantees as regards the local or general safety of Purevax FeLV vaccine in cats, as from the age of 8 weeks, administered in accordance with the vaccination schedule recommended by the manufacturer, and in the limit of the indications given under Side Effects in the Summary of Product Characteristics.

The data collected give evidence that vaccination of FeLV-infected animals is not harmful.

The safety of the vaccine has been supported by laboratory and field trials. Undesirable effects, their occurrence related to the time post vaccination and their duration are mentioned in the SPC.

No specific study was carried out in pregnant females. In the absence of such data, the vaccine is contra-indicated in this category of cats. No interference with the development of immunological functions has been observed.

No dissemination in the vaccinated animal was observed and no spreading of recombinant virus vaccine from vaccinated cats is expected. Given the non-replicative properties of the recombinant virus in cats, reversion to virulence was not investigated. Safety for canaries, guinea-pigs, mice and poultry has been demonstrated. However, the evaluation of the possibility of in vivo recombination with a field virus was performed with another recombinant virus.

No interaction has been observed when Purevax FeLV is administered simultaneously, but via a separate site, with Merial attenuated vaccine against feline panleukopenia and inactivated vaccines against feline viral rhinotracheitis, calicivirosis, chlamydiosis and rabies.

Field studies confirm the side effects observed in the laboratory trials. No ecotoxicity is expected.

In conclusion, the safety of the product has been demonstrated and the side effects are clearly mentioned in the SPC.

OVERVIEW OF PART IV OF THE DOSSIER: EFFICACY

INTRODUCTION

Purevax FeLV is a novel vaccine intended for the active immunisation of cats against feline leukaemia virus (FeLV) for the prevention of persistent viraemia and diseases associated with FeLV infections. The active component of the vaccine is a live recombinant canarypox virus vector in which are inserted appropriate genes of FeLV of subgroup A. The vaccine is either freeze dried and reconstituted before use with diluent or presented as a suspension for injection. Purevax FeLV is intended for the immunisation of kittens from eight weeks of age and is to be administered by subcutaneous inoculation in a dose of 1ml. A second dose should be given three to five weeks later. Duration of immunity has only been established one year after primary vaccination and therefore an annual booster dose of vaccine is recommended.

The dossier on efficacy presented by the manufacturer is based upon laboratory trials using two challenge systems: first, an oronasal challenge system that mimics natural exposure; and secondly, a system that closely simulates transmission in the field by exposing vaccinated kittens to viraemic cats that are excreting virus. The results are presented in five reports.

Justification for the vaccine

FeLV is a common infection of domestic cats throughout the world and a cause of significant morbidity and mortality. In European countries, several different FeLV vaccines are available all of which comprise inactivated virus or recombinant subunits. Efficacy of 70-100% has been claimed for these vaccines in experimental infections (reviewed in Sparkes, 1997) but little is known of their efficacy in the field. Since apparent vaccine breakdowns have been observed, there is clearly a need for improvement in vaccine efficacy and particularly for more evidence of protection in field conditions.

Purevax FeLV contains a live recombinant canarypox-FeLV virus in which appropriate FeLV genes have been inserted). The expression of this gene should stimulate an immune response to FeLV proteins. While it is recognised that virus neutralising antibodies are an important indicator of protection against FeLV infection, it is widely believed that cell mediated immunity plays a major role in natural recovery from infection and in vaccinal immunity. In fact, none of the currently available vaccines consistently induces virus neutralising antibodies following vaccination. Another rationale for the use of a live virus vaccine for FeLV is that the expressed proteins are presented to the immune system by antigen presenting cells through the endogenous processing pathway; therefore protective cell mediated immunity is very likely to be induced. A particular advantage of using canarypox virus as a vector is that it undergoes an abortive infection in mammalian cells so that no progeny virus is made and therefore the vaccine virus cannot spread from vaccinated animals.

General features of the design of FeLV vaccine trials

In the trials of Purevax FeLV, the manufacturers have used a challenge by either a single oronasal administration of virus (laboratory trials) or by exposure of vaccinated cats and appropriate controls to cats already excreting virus (field trial). In the latter case, a method was evolved in which a very high proportion of non-vaccinated cats became persistently viraemic and allowed significant protection of the vaccine to be demonstrated.

Throughout the dossier, the company has adhered to the requirements of the European Pharmacopoeia for inactivated vaccines although clearly the product is not inactivated. However, there are several characteristics of Purevax FeLV which indicate that it is not unreasonable to consider this novel vaccine in the context of these requirements.

GENERAL REQUIREMENTS

Choice of FeLV genes

The general requirements of the vaccine are set out clearly in the dossier. The logic for the choice of FeLV genes to be incorporated into the canarypox vector is valid.

Data required from laboratory and field trials on:

- **Each category of each target species**
  Challenges were performed on animals vaccinated at the age of 7 to 10 weeks.
- **Each recommended route of administration**
  All animals were vaccinated by the subcutaneous injection
- **Proposed schedule of administration**
  All animals were vaccinated according to the scheme of administration
- **Effect of passively acquired and maternally derived antibodies**
  Effect of passively acquired and maternally derived antibodies were not investigated
- **Claims regarding onset and duration of protection**
  No claim is made on the onset of immunity. Challenges were performed 2 weeks after vaccination. The annual booster vaccination is supported by a challenge performed more than one year after vaccination.
- **Each component**
  The vaccine contains one component only.

LABORATORY TRIALS

Dose response experiments

A dose response study was undertaken to determine the minimum dose of the recombinant virus required for protection against an appropriate FeLV challengeSpecific pathogen free kittens of 8-10 weeks of age were vaccinated on two occasions, 3 weeks apart with various doses of vCP97 canarypox recombinant virus and then challenged 2 weeks after the second dose of vaccine with FeLV-A given by the oronasal route to simulate natural infection.
The kittens were then monitored at intervals of three weeks for 12 weeks to determine their virus status. In the control groups, the challenge virus induced persistent infection in 83%.
The minimum calculated dose of recombinant virus to protect 80% of cats, thereby fulfilling the criteria of the European Pharmacopoeia, was $10^{7.24}$ $CCID_{50}$.

Potency test of vaccine

This trial was designed to establish the efficacy of the canarypox-FeLV vaccine administered in the dose previously found in the dose response trials to be required for significant protection against oronasal FeLV challenge. The vaccine was adjusted to provide $10^{7.5}$ $CCID_{50}$ in the inoculated dose. Three groups of specific pathogen free kittens were used. The kittens were then challenged 2 weeks after the second vaccination on day 42, when 13-15 weeks old, by the oronasal instillation of FeLV-A/Glasgow-1.

Anti-gp70 antibody in the sera of the cats was detected by a competition enzyme immunoassay. Surprisingly, not all virus-negative cats developed antibodies. Superficially there was a lower proportion of non-antigenaemic cats which developed anti-gp70 antibodies in Group 1 compared to the other groups (Group 1: 7/17, Group 2: 4/5, Group 3: 3/4). However, these differences are not statistically significant.

This trial demonstrated adequately that the FeLV vaccine RMB678 provided excellent protection against a vigorous laboratory challenge with FeLV.

Duration of immunity

The aim of this trial was to determine the efficacy of vaccination against FeLV using the recombinant canarypox-FeLV vaccine, one year after primary vaccination. The vaccine was used either alone or in combination with vaccines against other feline infectious diseases, produced by the company.

The cats were monitored after challenge for FeLV p27 antigen in the serum by a commercially available test. Testing began 3 weeks after challenge and continued at weekly intervals until week 15 after challenge in line with the requirements of the European Pharmacopoeia. Anti-gp70 antibodies were also monitored. None was present at the day of challenge. In the control groups, 67% of the older cats and 100% of the kittens were defined as being persistently infected opposed to less than 20% in the vaccinated animals.

In conclusion, this trial is a demonstration of the ability of the canarypox-FeLV vaccine to protect cats from FeLV challenge, more than one year after vaccination.

Protection against latency

The manufacturer has presented in the dossier a claim that Purevax FeLV prevents the establishment of latent FeLV infection following intranasal challenge of vaccinated cats with FeLV-A. Latency may be considered as a transitional state between infection and recovery.

The study was conducted under conditions that would have detected latent virus.. The enzyme immunoassay used to test for the release of FeLV antigen from the cell cultures established from the bone marrow cells appears to be sensitive in that in all cases where cultures from viraemic cats were tested, antigen was unequivocally demonstrable within one week of culture.

FIELD TRIALS

Efficacy under natural condition of challenge.

The aim of the trial in was to determine the efficacy of vaccination against a natural challenge of FeLV. This was achieved through exposure of vaccinated and appropriate control kittens to persistently viraemic and shedding kittens with which they were housed. In this way it was intended to simulate the effects of vaccination and infection in field conditions.

- Donor viraemic kittens (Group A) were obtained by infecting a group of 18 8-week old SPF kittens
- Two groups of 24 kittens were used as vaccinates:

• Group B comprised kittens vaccinated at 8-9 weeks of age and then four weeks later with the canarypox-FeLV recombinant virus.
• The kittens in Group C were vaccinated under with Competitor Product A the first dose being administered at 10 weeks of age and the second dose three weeks later.

- Two weeks after the second vaccination the vaccinated and control kittens were mixed with the kittens in Group A

The kittens were then monitored at intervals of three weeks for the presence of FeLV p27 antigen and infectious virus in the blood until 27-28 weeks after exposure to the viraemic kittens.

The vaccine gave excellent protection in the face of this rigorous challenge. The first evidence of viraemia occurred four weeks after exposure in kittens in the control group and later, at 6 weeks, in a few kittens in the vaccinated groups.

The Purevax FeLVvaccine gave excellent protection of kittens against a very severe natural challenge of FeLV.

CONCLUSIONS ON EFFICACY

The dossier provides a comprehensive account of the development of a novel vaccine against FeLV infection of the domestic cat. Reports of trials support the claim of the manufacturer that the vaccine, Purevax FeLV, provides excellent protection against FeLV challenge.

The design of the vaccine was logical. FeLV-A was chosen as a source of FeLV genes since it is present in all known isolates of the virus and immunity to FeLV-A should confer protection against all three subgroups of the virus. The env, gag, and part of the pol genes of FeLV were included in the vaccine since all were considered necessary for the induction of an adequate immune response to the virus. Canarypox virus was chosen as a vector since it leads to high level expression of inserted genes, presentation of antigens through the endogenous pathway and does

not spread from vaccinated animals. In designing trials of efficacy, the manufacturers adhered to the European Pharmacopoeia requirements for inactivated FeLV vaccines.. The trials were conducted in strict accordance with these requirements.

The experiments described in the dossier were carried out in a logical manner in order to determine the dose of canarypox recombinant virus necessary for use in the vaccine, to establish the potency of the vaccine against an appropriate laboratory challenge and to show efficacy against a severe natural challenge. The techniques used were standard for the subject and the statistical analyses of the results were valid.

The dose of virus ($10^{7.5}$ $CCID_{50}$) used in the vaccine was determined in dose-response trials using an intranasal challenge at a single time. The choice of the dose of vaccine virus was subsequently validated in the two laboratory trials and one field trial of efficacy.

The efficacy of the vaccine at that dose was established against a good challenge under laboratory conditions and the conditions satisfied the requirements of the European Pharmacopoeia. The duration of immunity study clearly demonstrated that the vaccine afforded excellent protection over a period of at least 12 months. This trial was conducted under difficult conditions because of the age-related resistance of cats to FeLV infection.

The trial, carried out under field conditions also gave an impressive confirmation of the strength of the protection afforded by the vaccine. Previously others had found difficulty in designing trials to show efficacy under these conditions. In this case, a sufficiently high challenge was achieved in the unvaccinated cats to reveal excellent protection by the vaccine.

The applicant claims that the vaccine protects against FeLV infection and FeLV-associated diseases. Whilst the trials described in the dossier were not carried out in a time frame that would allow the development of most FeLV-related diseases, which have an incubation period of several years, it is generally agreed that cats which recover from challenge with FeLV and are non-viraemic have an equivalent disease risk to cats that have never been exposed to the virus (McClelland et al., 1980). Therefore this claim is considered valid.

The efficacy was established in both laboratory trials using a challenge method that simulates natural infection, and in conditions of natural challenge through exposure to excreting cats. Therefore the claim of the manufacturer is valid. Purevax FeLV has been tested on kittens vaccinated from 8 weeks, the minimum age recommended for vaccination, according to the scheme of vaccination. The efficacy of the vaccine in cats vaccinated at the adult age has not been tested.
All the laboratory trials have been performed using a vaccine virus dose lower or equal to the minimal recommended vaccine dose. A minimal dose of $10^{7.24}$, lower than the recommended vaccine dose, to protect 80% of the vaccinated animals against persistent antigenemia has been established.
A challenge using an oronasal route and a virulent strain was used. The challenge failed to induce persistent viraemia in 80% of unvaccinated kittens. However, the same challenge induced 100% viraemia in another trial.

The duration of immunity of one year is supported.

In conclusion, the efficacy of the vaccine has been proven in laboratory conditions, showing an active immunisation against feline leukaemia for the prevention of persistent viraemia.

RISK-BENEFIT ASSESSMENT AND CONCLUSION

FeLV is a common infection of domestic cats throughout the world and a cause of significant morbidity and mortality. In European countries, several different FeLV vaccines are available all of which comprise inactivated virus or recombinant subunits. Efficacy of 70-100% has been claimed for these vaccines in experimental infections (reviewed in Sparkes, 1997) but little is known of their efficacy in the field. Since apparent vaccine breakdowns have been observed, there is clearly a need for improvement in vaccine efficacy and particularly for more evidence of protection in field conditions.

Purevax FeLV contains a live recombinant canarypox-FeLV virus in which appropriate FeLV genes have been inserted). The expression of this gene should stimulate an immune response to FeLV proteins. While it is recognised that virus neutralising antibodies are an important indicator of protection against FeLV infection, it is widely believed that cell mediated immunity plays a major role in natural recovery from infection and in vaccinal immunity.

The efficacy was established in both laboratory trials using a challenge method that simulates natural infection, and in conditions of natural challenge through exposure to excreting cats.

Additional data show the very limited risk of spread into the environment, as well as the absence of known risk of amplification in mammals due to the defective vector and, more specifically, the absence of risk in humans. The data supplied by the manufacturer for a risk analysis and complemented by available scientific data are mainly based on the defective character of the vaccine strain for replication in mammals, in particular in cats and humans.

Further to an oral explanation provided by the applicant, it is considered that Purevax FeLV does not show any documented risk. Moreover, based on current scientific knowledge and virological concepts, no foreseeable risk is associated with its use under the conditions described. Furthermore, the data presented by the company in its dossier are in accordance with the Scientific Advice provided by the CVMP.

Based on the original and complementary data presented, the Committee for Veterinary Medicinal Products concluded that the quality, safety and efficacy of the product were considered to be in accordance with the requirements of Council Directive 81/852/EEC as amended (now Directive 2001/82/EC) and supported the claims proposed by the Applicant.