SCIENTIFIC DISCUSSION

1. INTRODUCTION

Fevaxyn Pentofel is a pentavalent feline vaccine containing four inactivated viral antigens and one inactivated chlamydial antigen. The formulation includes an adjuvant system and is presented as a preservative-free aqueous solution in single-dose syringes.

The formulation proposed for marketing was previously marketed in Spain only. This marketing authorisation was, however, withdrawn on 17 May 1995, as Fort Dodge applied to the EMEA for the granting of a Community marketing authorisation. Outside the European Union, the formulation proposed for marketing had not been marketed previously. Each of the components of the vaccine had, however, already appeared in other marketed formulations in the EU and/or the United States. The most relevant of these is Fel-O-Vax Lvk IV which is licensed in the United States and which contains the same active ingredients and adjuvant system as Fevaxyn Pentofel, but differs from it as it also contains thiomersal and EDTA as excipients.

The vaccine is contained in 3ml polypropylene syringes, 1 dose per pre-filled syringe, closed with a rubber tip. The syringes are packed in cardboard cartons in quantities of 10, 20 or 25.

Fevaxyn Pentofel qualifies as a biotechnological medicinal product under Part A of the Annex to Council Regulation (EEC) No 2309/93 because the Feline Leukaemia Virus component is produced in a persistently provirus-infected prokaryotic cell line which had been established using techniques which involved cleaving DNA from the thymus of an infected cat, sub-cloning into a recombinant vector and subsequent transfection into a feline fibroblast cell-line.

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

2.1 Qualitative and quantitative particulars of the constituents

The quantitative composition, which was originally insufficiently stated, was satisfactorily presented as potency. The ingredients of the adjuvant and other excipients were stated in the composition.

The Committee sought clarification on whether immunological studies had been conducted, which documented the choice of vaccine strains for the inactivated vaccine. Immunogenicity studies demonstrating the suitability of each of the five strains in Fevaxyn Pentofel were conducted and all of them were carried out with the combination product; as it had been agreed that studies using the combination product, which was to be marketed, were appropriate. Early doubts that the Feline Panleukopenia product fraction did not meet the specifications of the European Pharmacopoeia were resolved; as the applicant supplied further data which confirmed compliance with the immunogenicity criteria stated in the European Pharmacopoeia monograph.

The medium used in cell passaging and bioreactor production was changed, so that any bovine material was sourced from the USA and New Zealand (declared free of Bovine Spongiform Encephalopathy) and inactivated by irradiation. Thiomersal and EDTA, which were originally included as preservatives, were no longer required in the current single dose containers and were not, therefore, included in the blend stage during manufacture of the product. Such changes were shown to have no adverse effects on the safety or efficacy of the product. Thiomersal was only used as an inactivant of the feline *Chlamydia psittaci* component. Minor changes in the volume of the inactivant and the diluent were confirmed as having no effect on the safety or efficacy of the product.

2.2 Method of preparation

The Committee expressed concerns relating to the production of the vaccine at the manufacturing site in Ireland. Specifically, information was requested on how to avoid the potential risk for cross contamination of the ventilation system between different campaign productions, and measures were required to ensure cleaning validation between campaign production. Documentation relative to all these points was made available in the Plant Master File for the manufacturing site; to the satisfaction of the CVMP.

A process of ultrafiltration of antigen stocks is undertaken to reduce the volume of stocks and hence increase their potency. As Fevaxyn Pentofel is a five antigen product, contained in a 1ml dose, it is not possible to produce sufficiently potent antigen stocks, of four of the five fractions in Fevaxyn Pentofel, to blend into a 1 ml dose without ultrafiltration. Feline Calicivirus (FCV) is the only antigen stock which is not routinely concentrated. For all antigens, each stock must meet an established Relative Potency, both pre and post concentration (except for FCV). For each of the antigen stocks, there is an established concentration factor determined from experimental studies where it has been demonstrated that when each of the antigen stocks achieves the minimum relative potency, as declared in the composition of the product, the finished product is efficacious in cats.

In 1999, the applicant applied for a variation to introduce an additional manufacturing site for the Feline Leukaemia Virus antigen: in Charles City, Iowa, USA. Refinements to the FeLV antigen production methods were also proposed for both production sites. This variation was accepted by the CVMP on November 1999.

2.3 Control of starting materials

When initially reviewing the data presented on control of starting materials, the Committee expressed its reservations on the apparent lack of information on tests on each material of biological origin. The applicant was able to demonstrate that a validated irradiation process, to gamma irradiate all products of animal origin, was utilised, as well as testing at the pre- irradiation stage to ensure freedom from extraneous agents. However, testing for extraneous agents was carried out according to 9 CFR requirements and not according to equivalent test methods described in the CVMP guidelines on inactivated vaccines. Whilst the Committee accepted that the four seed viruses, the Chlamydiae and the two cell lines (MDCK and CRFK) had all been tested extensively using 9 CFR methods, which appear to be dependable and established internationally, testing should still be undertaken according to CVMP guidelines within a set time frame - by the end of 1996. However, this requirement did not delay the authorisation and the Applicant addressed this request later on as follow-up measures. It was confirmed that routine testing for mycoplasmas was conducted according to the requirements of the European Pharmacopoeia.

Assurances were also provided by the applicant, to the satisfaction of the Committee, that materials of ruminant origin would meet the requirements laid down in the CVMP Spongiform Encephalopathies guideline. All such materials are sourced from countries declared free of BSE. Furthermore, assurances were provided that no ruminants in these countries are fed with foodstuffs containing ruminant proteins derived from offal.

The applicant provided an identification assay for two of the three constituants of the adjuvant, and undertook to make every effort to develop an identification assay for the third component, Neocryl, as a starting material. The Committee agreed that this should not delay authorisation and the Applicant addressed this issue satisfactorily as follow-up measures in January 1997.

The Committee's requirement, that each of the master seed components should be tested for immunogenicity, was satisfied by the applicants assurances that such testing is carried out using the formulation and method of manufacturing to be used for the marketed product. Furthermore, the CVMP agreed that it was appropriate that, for a multivalent vaccine, the immunogenicity of each of the vaccine seeds was investigated in the presence of the other components of the final formulation.

The Committee was assured on methods employed in the in-process control. The applicant uses relative potency values (RP), measured by ELISA as an alternative in-process control to pre-inactivation titres, to determine the antigen content of each antigen stock and to provide test results for blending each batch of Fevaxyn Pentofel. Antigen stocks are accepted for use if they meet the relative potency RP values stated on the antigen stock specifications. Stocks which meet the specifications may be used in the product blend with additional volume controls and prototype testing. The antigen content of each blend is then verified by the validated *in vivo* potency test in cats.

In 2001 the applicant applied, by a Type I variation, to amend the potency test for the Chlamydia component. A commercial test kit was to be replaced with an in-house method; both tests being ELISA capture methods. Validation of the proposed test method was satisfactorily demonstrated, including evaluation of precision, linearity and specificity. At the request of the CVMP, the prototype batches used for the validation were shown by the applicant to be equivalent to the regular batches. The two ELISA methods were evaluated as being equivalent. The specification for batch release was unchanged. This variation was accepted by the CVMP in November 2001.

2.4 Control of the finished product

In final product testing, the currently available information on the reference vaccine used in ELISA analysis had been considered to be inadequate in terms of a full analysis and the criteria to be identified to establish a new reference. Currently, the reference vaccine is formulated in an identical manner to Fevaxyn Pentofel routine production batches and blended to contain minimal immunogenic quantities of each antigen stock so that batches of antigens tested against the reference vaccine will meet predetermined relative potency values at the blend stage. The applicant, however, recognised the need to qualify a new reference vaccine following WHO guidelines for its preparation and committed to submit a full report on the new reference vaccine by December 1996; this did not delay the authorisation and the Appplicant fulfilled his commitment later on.

The applicant clarified that 5 SPF cats are used to test the serological response to vaccination with the Feline Panleukopenia virus, with the Feline Calicivirus, with the Feline Rhinotracheitis virus and with the Feline Chlamydia psittaci. In this test, 4 cats are vaccinated and one is used as an environmental control. The same 5 cats and one additional cat are then used for the feline leukaemia challenge test. The criteria for the antibody-titre release of batches in the cat in vivo test were satisfactory.

For various reasons (in particular animal welfare) the applicant is willing to replace the *in vivo* potency test by an *in vitro* method. However, the correlation between the *in vivo* potency results and the ELISA assay results must be established first. Therefore, the company undertook to make an analysis of the correlation of the ELISA assay results on the finished product with the *in vivo* cat potency test results for three batches. As a result of satisfactory correlation between the two, Fort Dodge Laboratories later amended the release potency test to the *in vitro* assay by variation. This variation was accepted by the CVMP on September 1998.

The abnormal toxicity test originally proposed by the applicant on the final product was no longer required by the European Pharmacopoeia because of unnecessary use of large numbers of animals to little benefit. The Committee recommended that the test should no longer be used and the applicant was to replace it with a safety test in the target animal, at the product blend stage.

2.5 Stability

In accordance with CVMP guidelines on stability testing for inactivated vaccines, a short temporary shelf life of one year at +2º - +8ºC was agreed, pending the completion of a real time stability study on 3 full scale production batches for 24 months at +2º - +8ºC. These studies were to begin in the third quarter of 1996.

The Committee could not agree, however, to the applicants request that stability data on antigen stocks were not to be submitted, particularly when a 2 year period of storage was proposed for such stocks. The applicant was, therefore, required to pursue stability studies in antigen stocks and provide data on an on-going basis.

3. OVERVIEW OF PART III OF THE DOSSIER: TOXICOLOGICAL AND PHARMACOLOGICAL ASPECTS

3.1 Laboratory tests

The laboratory safety tests undertaken include : administration of one dose, administration of an overdose and repeated administration of one dose. These tests were conducted in the target species, and the animals were examined daily for 14 days, following vaccination, for signs of local and systemic reactions, daily temperature and other performance measurements. The results of these tests were satisfactory although the batch of vaccine used for these safety tests had Relative Potency values for some of the antigenic components which were less than those required at the end of the shelf life.

More importantly, studies on the safety of the vaccine were initially conducted on 12-week old kittens and not at the minimum of eight weeks recommended for vaccination. Also, the safety of the vaccine in pregnant queens has not been demonstrated, although no cases of abortion or impaired reproductive performance are reported in the pharmacovigilance data presented in the dossier.

Consequently, the applicant has initially withdrawn the indication for vaccination of eight week old kittens and pregnant queens. The Applicant has later applied for a variation providing further data to amend the minimum age of first vaccination for the target species from 12 weeks to 9 weeks of age. Vaccination at 9 weeks of age is recommended for cats living in high risk environment. Such animals should be advised a primary vaccination of 3 injections 3 weeks apart starting at 9 weeks of age. This variation was accepted by the CVMP in August 1999.

Additional studies concerning specific safety aspects of the FeLV virus strain contained in the vaccine have also been carried out. In particular, the potential immunosuppressive effect, by examining T-cell function in vaccinated cats, has been evaluated, and the safety of the vaccine when used in cats with persistent FeLV viraemia at the time of vaccination, has been assessed.

Immunosuppressive effect

The Committee also discussed the possible immunosuppressive effect of the Feline Leukaemia antigen potentially resulting from the presence of the envelope protein p15E. As the tests for measuring potential immunosuppressive effects were not carried out with the final product and as the follow-up period was not long enough (only 4 days), the Committee agreed that the data concerning the absence of immunosuppressive properties of the vaccine were insufficient and that additional information be provided by the applicant. Test results from day 7, 14, and 21 were requested.

A new study was subsequently conducted by the applicant with thirty, eight-week old male SPF-kittens seronegative to the five antigens of the vaccine. The results of the study provided no evidence that vaccination would have immunosuppressive effects.

Vaccination of cats with persistent FeLV viraemia

The vaccination of cats with persistent FeLV viraemia was intended to illustrate that it did not exacerbate the pre-existing infection or had any safety implications for vaccinates. The Committee however considered that the follow-up period after vaccination was too short (12 weeks) to enable valid conclusions.

As the applicant did not wish to claim that the vaccine would alter the course of feline leukaemia disease in cats with persistent viraemia, and given that the vaccine is intended for healthy cats only, the Committee decided not to pursue the matter further, agreeing that no further studies were necessary.

3.2 Field studies

A total of 416 cats of various breeds were included in the safety trials carried out in the United States. The trials were designed to observe the occurrence of any untoward post-vaccinal reactions. Overall, there were no reactions in 94.2% and 99% following first and second vaccinations respectively. All observed reactions in the remaining cats were transitory.

These results were further supported by pharmacovigilance data collected in the US since 1991 when the vaccine was first licensed, and where several million doses have been sold since then.

The results of the field trial and the pharmacovigilance data were considered as valid evidence by the Committee that few untoward post-vaccinal reactions could be expected to arise in connection with use of the vaccine under practical conditions.

3.3 Ecotoxicity

No ecotoxicity studies were reported. The Committee considered this acceptable since the vaccine in question is inactivated, hence the phenomenon of transmissibility does not arise. Furthermore, the route of administration (subcutaneous injection) coupled with the method of dispensing in single vial doses, diminishes any threat to the environment to negligible proportions.

4. OVERVIEW OF PART IV OF THE DOSSIER: CLINICAL ASPECTS

4.1 General Requirements

An immunogenicity and antigen interference study was carried out to evaluate the efficacy of the five antigens of the vaccine and to evaluate possible antigen blockage occurring. Four of the five agents present in the vaccine were used in the challenge experiments. The exception was Feline Panleucopenia virus, where the measurement of protective serum neutralising antibodies was used as a substitute for challenge.

The duration of immunity for all the vaccine agents has been evaluated in another study, employing challenge with all agents.

Though these experimental studies have not been carried out under GLP conditions, the Committee considered that, as regards their design, execution, and reporting, these studies were of a quality not hindering valid conclusions to be drawn with respect to efficacy, based on the reported results.

4.2 Laboratory trials

Immunogenicity and Antigen Interference

For the FeLV fraction, the vaccine has induced protection of considerable magnitude against FeLV challenge, under the conditions of the experiment. The Committee considered that these results indicated a satisfactory degree of protection.

The applicant provided satisfactory evidence that the vaccine will also induce protection against upper respiratory disease of cats caused by Feline Calicivirus, Feline Rhinotracheitis Virus and Chlamydia psittaci, and against disease caused by Feline Panleukopenia Virus.

The Committee noted that this vaccine was not protective against shedding of Chlamydia psittaci, which is a common feature to similar existing vaccines.

Overall the Committee concluded that the efficacy of the individual fractions of the vaccine were satisfactory and that there was no evidence of antigenic interference.

Duration of Immunity and Re-vaccination

To study the duration of immunity, vaccinates were challenged approximately one year after vaccination according to recommendations.

With the exception of the Feline Calicivirus Fraction, where results indicate that protection may decline significantly over one year and support the annual re-vaccination proposed by the applicant, all four other fractions were shown to have induced a significant level of protection, not necessarily requiring an annual re-vaccination.

Although the results do not strongly support the applicant's recommendation for an annual re-vaccination, the Committee nonetheless followed the applicant's proposal of an annual re-vaccination, considering that it was the best compromise for a multicomponent vaccine like Fevaxyn Pentofel.

Protection against Tumour Occurrence

A claim for protection against tumour occurrence had not been made by the company in the original application. However, since formation of tumours (lymphosarcomas) is one of the main manifestations of leukemia, the Committee considered that it was reasonable to require that the protection afforded by a vaccine against leukaemia should include protection against tumour formation. Therefore, the Committee agreed that the applicant should be asked to provide evidence as to whether the vaccine would reduce the emergence of tumours (lymphosarcomas).

In its response, the applicant stated that the persistent viraemia was associated with an 80 percent risk of death within the following three years, either because of lymphosarcoma (approximately one-third of the cases), or due to diseases connected with the immunosuppressive effect of the virus, and furthermore concluded that Pentofel by protecting vaccinates against persistent viraemia, would provide a significant level of protection against tumour formation and immunosuppression.

The Committee accepted the explanation provided by the applicant.

4.3 Field trials

Recognising that it would be extremely difficult to obtain field data of a quality that would allow valid conclusions to be drawn concerning the protective effect of the vaccine when used under practical conditions, the Committee considered acceptable that no study concerning the protective effect of the vaccine when used under field conditions had been carried out. In addition, with the exception of the vaccine's FeLV fraction, the Committee considered that sufficient experience with vaccine agents have already been published, to allow valid conclusions to be drawn concerning the vaccine's performance in the field, based on the experimental data contained in the documentation submitted by the applicant.

In addition, the Committee stated that the product should not be used on pregnant cats, except if the claim could be substantiated.

5. CONCLUSION

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

Some minor quality points still needed to be clarified at the time of the authorisation (e.g. stability data, extraneous agents testing, correlation between in vivo and vitro potency results). However, the Committee agreed that these could be addressed on an on on-going basis by the applicant without delaying the authorisation process. The applicant agreed to provide within the defined timeframe the data requested by the Committee and all commitments have been satisfactorily addressed later on. Consequently, the Committee recommended on 18 September 1996 that the product could be recommended for the granting of a Community marketing authorisation.