1. SUMMARY OF THE DOSSIER

Netvax is a vaccine intended for use in chickens to provide passive protection to chicks against necrotic enteritis (NE). It is an inactivated vaccine containing a toxoid of *Clostridium perfringens* type A alpha toxin combined with an oil adjuvant.

Necrotic diseases in domestic chickens are associated with *Clostridium perfringens* type A. It is common in the gut of chicks and is found in the general poultry production environment. It has been identified as occurring as early as 2 weeks of age. Necrotic enteritis is a multi-factorial disease characterised by reduced performance of broiler birds and increase in mortality during the production cycle.

The vaccination schedule consists of two doses: the first dose is given at 10 to 14 weeks of age, and then a second dose is given 4 to 10 weeks after the first. This second dose should be given at least six weeks before the hen will start to lay eggs. The onset of passive transfer of immunity is 6 weeks following completion of the vaccination procedure. The duration of passive transfer of immunity is 51 weeks following completion of the vaccination procedure.

The benefits of Netvax are the active immunisation of chickens in order to provide passive immunisation against necrotic enteritis to their progeny, during the laying period and the reduction of mortality and the incidence and severity of lesions caused by *Clostridium perfringens* Type A induced necrotic enteritis. The most common side effect is a moderate swelling of the breast tissue which will resolve within 30 days. Following the second vaccination swelling may persist for at least 35 days.

2. QUALITY ASSESSMENT

COMPOSITION

On dose of 0.5 ml contains:

<table>
<thead>
<tr>
<th>Name of Ingredients</th>
<th>Quantity per 0.5ml dose</th>
<th>Reference to Standards</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Active Ingredients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Clostridium</em> perfringens alpha toxoid derived from Type A</td>
<td>Not less than 3 TCP units</td>
<td>IU</td>
</tr>
<tr>
<td><strong>Constituents of Excipients</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium Chloride (0.85%w/v)</td>
<td></td>
<td>Ph Eur</td>
</tr>
<tr>
<td>Formaldehyde</td>
<td></td>
<td>Ph Eur</td>
</tr>
<tr>
<td>Thiomersal 0.035-0.05 mg</td>
<td></td>
<td>EP</td>
</tr>
<tr>
<td>EDTA</td>
<td></td>
<td>In House</td>
</tr>
<tr>
<td><strong>Constituents of Adjuvant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light Mineral Oil (Draked 6VR)</td>
<td>0.31 ml</td>
<td>In House</td>
</tr>
<tr>
<td>Sorbitan Oleate</td>
<td></td>
<td>Ph Eur</td>
</tr>
<tr>
<td>Polysorbate 80</td>
<td></td>
<td>Ph Eur</td>
</tr>
<tr>
<td>Benzyl Alcohol</td>
<td></td>
<td>Ph Eur</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td></td>
<td>Ph Eur</td>
</tr>
</tbody>
</table>

*TCP= Total Combining Power. The product is released based on rabbit potency. The upper limit is 4 TCP/dose.*

Medicinal product no longer authorised
CONTAINER
The vaccine is filled into 500 ml high density polyethylene (HDPE) flexible bottles that are designed
to collapse as the product is removed. The material complies with Ph. Eur. chapter 3.1.4.
Bottles are closed with 20 mm chlorobutyl rubber closures made from material that complies with Ph.
Eur. chapter 3.2.9. The closures are treated with silicone oil that complies with Ph. Eur. chapter 3.1.8.
The closures are completed with 20 mm aluminium seals with a centre hole. Plastic bottles, closures
and seals are sterilised by gamma irradiation.
Specifications and certificates were provided.

DEVELOPMENT PHARMACEUTICS
The Clostridium perfringens type A alpha toxoid antigen included in this vaccine is manufactured by
Schering-Plough Animal Health, Upper Hutt, New Zealand and is already used as a component of an
existing product for sheep and cattle, Covexin 10. The strain produces high levels of alpha toxin
which is inactivated to form the active ingredient in this vaccine. The origin of the isolate is unknown
but the alpha toxin is highly conserved from strain to strain, with almost identical sequence and
biochemical properties.

Selection of Adjuvant
Presentation of the antigen in a suitable carrier is known to enhance the stimulation of immunological
responses. Oil adjuvants are one vehicle used to achieve this. The presence of the adjuvant allows
delayed release of the antigen at the site of injection and enhances uptake of the antigen by
immunological cells. The Applicant carried out a number of studies during the development of Netvax
to select a suitable adjuvant and a suitable dose of C. perfringens Type A alpha toxoid in the chicken.
The adjuvant consists of light mineral oil, sorbitan oleate, polysorbate 80, benzyl alcohol and
triethanolamine.

Active ingredient
The Applicant carried out a dose titration study to select a suitable antigen input for the vaccine:
Ten-week-old female white leghorn SPF chickens were used. Three different vaccines were selected
and specifically blended to contain 1, 2 or 3 TCP of C. perfringens type A toxoid. All contained
Drakeol adjuvant. Three groups of chickens were vaccinated with one of the above vaccines and a
fourth group was kept as controls.
There was a dose related antibody response to vaccination and a dose of 2 TCP per 0.5ml dose was
selected as the lowest dose to be tested in vaccination/challenge experiments with Netvax.

Excipients
Sodium chloride solution is used as a diluent when blending the vaccine.
Formaldehyde is used during manufacturing to inactivate the clostridial organism and detoxify the
toxin.

Container
The vaccine is administered parenterally and in normal use it is administered to several animals on the
same occasion. It is therefore presented in a sterile, multidose container.

Preservative
In compliance with the pharmacopoeial requirement for a preservative to be included in multidose
presentations, thiomersal is included in the final vaccine formulation. Thiomersal is included in Annex
II of Council Regulation [EEC] No 2377/90 and is permitted to be used for this purpose in multidose
containers at a concentration of not more than 0.02% (w/v) without the need for establishing a
maximum residue limit or a withdrawal time.
Composition of the batches used in the clinical trials
Details of batches of vaccine used in laboratory and field trials were provided along with the respective production records. Studies designed to examine the effect of scale of production on the immune response and to show no significant difference between batches produced at different sites were presented and were found acceptable.

METHOD OF MANUFACTURE
A flow chart indicating the production process for the *C. perfringens* antigen and a more detailed description were provided. In summary, the product is manufactured as follows: The active ingredient of the vaccine, *C. perfringens* type A alpha toxoid, is produced by formaldehyde treatment of the toxin produced during culture of *C. perfringens* strain CN 1491. After inactivation of the culture using formaldehyde, bacterial cells are removed by centrifugation, the supernatant is concentrated by ultrafiltration and toxoiding is continued. If residual toxicity remains at this time, toxoiding may be continued with or without addition of more formaldehyde. To prepare the final product, a measured quantity of toxoid (target 3 TCP/dose) is blended with a mineral oil adjuvant to form a water-in-oil emulsion. The process was reasonably well described.

The Applicant accepted that the antigen should be stored for no longer than 12 months and committed to provide data relating to stability of the final product blended with antigen stored for 12 months.

A flow chart of the blending process to produce the finished vaccine was provided with a detailed description. Adjuvant, antigen and excipients were combined to form an oil emulsion which is filled into final containers. Satisfactory details on the emulsification time were provided which were considered acceptable.

Validation studies
Inactivation and toxoiding of *Clostridium perfringens* type A
The treatment of *C. perfringens* type A with formaldehyde solution served two purposes: a) to inactivate the bacterial cells and b) to convert the bacterial toxin to harmless toxoid. These two processes took place simultaneously but the inactivation was completed far more quickly than the toxoiding.

The current inactivation and toxoiding conditions for *Clostridium perfringens* Type A are the addition of formaldehyde. An inactivation study was carried out on 6 full scale commercial batches of *Clostridium perfringens* Type A.

Study - Inactivation kinetics report, *C. perfringens* Type A

Inactivation kinetics trials were carried out for 6 individual batches of *C. perfringens* type A. Samples were taken to test for complete inactivation before addition of formaldehyde and at 0 hrs, and after addition of formaldehyde at 10%, 33%, 67% and 100% of the inactivation period respectively. In the case of all batches, inactivation was complete within 10% of the specified inactivation period, which is within the maximum of the total inactivation time specified by the Ph. Eur.

More inactivation kinetics were performed on further batches of *C. perfringens* A with higher pre-inactivation titres. The results of these studies were presented and were found acceptable.

After the inactivation period, the bacteria are removed by centrifugation and, following the addition of more formaldehyde, toxoiding is continued for a further period before testing for non-toxicity. Due to the fact that the toxoiding process has been found to be quite variable, specific toxoiding kinetics studies have not been carried out but the process is adequately controlled by repeated in-process and final product freedom-from-toxicity tests to ensure that the toxoid used in the final product is safe.

Non-viability testing and limits of detection
Two trials were carried out to select the best medium to use for the test and to determine the sensitivity of the selected method in the presence and absence of formaldehyde. Two media were tested and the most sensitive one was selected for use in the non-viability test.
Continuous Steriliser
Validation data for the continuous sterilisation method applied to media was presented. It was concluded that the process is adequate to ensure the sterility of the media.

Concentration process
Although, the validation of this cleaning procedure is a GMP issue the CVMP considered that the inclusion of the validation gave added reassurance that the \textit{C. perfringens} toxoid used for this vaccine will not be contaminated with other clostridial antigens produced in the same facility and concentrated using the same filtration equipment.

Validation of antigen storage
A stability study on three batches of \textit{C. perfringens} Type A antigen is currently ongoing and a summary of results up to 18 months storage was presented. Parameters studied were the Total Combining Power, Non-Toxicity and Non-Viability. Requirements for Non-Toxicity and Non-Viability were met throughout the 18 months period.
It was concluded that the data can support an expiry of 12 months.

CONTROL OF STARTING MATERIALS

Listed in a Pharmacopoeia
The starting materials complying with the Ph.Eur are listed below:
Sodium chloride, Sodium hydroxide, Hydrochloric acid, Dextrin, Thiomersal, Benzyl alcohol, Trolamine, Polysorbate 80, Sorbitan oleate, Purified water.
Specifications and Certificates of analysis which demonstrate starting materials to be in compliance with Ph. Eur requirements were submitted.

Not listed in a Pharmacopoeia

\textbf{Starting materials of biological origin}
- \textit{Clostridium perfringens} type A, strain CN 1491
The Master Seed has been tested for identity and purity. It was noted that the Master Seed was prepared in 1974 so the risk that it could be contaminated with BSE agent is negligible.

The preparation of the workingseed was adequately described. They are tested for purity and identity, and fermentation trials may be carried out to confirm they meet the required toxin yield. Once testing is complete, they are referred to as Working Seeds and can be used for antigen production.

The material specifications and sample certificates of analysis for the rest of starting materials of biological origin were provided and were found to meet all the appropriate requirements. They are summarised below:
- Pancreatin 6NF
- Beef
- Liver extract
- Yeast extract
- Protease Alkaline

\textbf{Starting materials of non-biological origin}
The raw material specifications and a sample certificate of analysis for the non-biological starting materials below were provided. They were found to meet all required specifications.

\textbf{In House preparation of media}
The ingredients of all in house preparation media were described together with the methods of preparation. They were found acceptable and provided reassurance regarding their quality.
SPECIFIC MEASURES CONCERNING THE PREVENTION OF THE TRANSMISSION OF ANIMAL SPONGIFORM ENCEPHALOPATHIES

The TSE-Risk assessment for Netvax includes starting materials of animal origin used in the production of this vaccine such as beef, pancreatin 6NF, bovine liver digest (liver extract). Relevant data were provided and it was concluded that they constitute a low risk of contamination, either because their countries of origin are considered to be free of TSE infections or because the tissues they are sourced from are considered to have a low risk irrespective of the county of origin. A risk assessment for the bacterial master and working seed stocks of Clostridium perfringens Type A seed materials was provided and the risk was found negligible. In conclusion all starting materials of animal origin used in the production of the final product of Netvax were found to comply with the current regulatory texts related to the TSE Note for Guidance (EMEA/410/01-Rev.2) and Commission Directive 1999/104/EEC. Therefore, it was concluded that the vaccine does not pose a risk to vaccinated animals.

CONTROL TESTS DURING PRODUCTION

The stages at which the various tests are carried out during production were identified and a relevant flow chart was provided. The tests were found to be satisfactory for controlling the production processes and for ensuring consistency of production. The following tests are performed during production:

- Test for purity
- Measurement of pH
- Estimation of Total Combining Power (TCP)
- Test for Non-Toxicity
- Test for Non-Viability
- Test for Sterility

Production records from three antigen batches
The production records and test results for three consecutive batches of C. perfringens Type A alpha toxoid were provided. The three batches all were generally satisfactory and met the requirements needed to produce the finished product.

CONTROL TESTS ON THE FINISHED PRODUCT

General characteristics of the finished products
Specifications and controls that take place on the finished product were provided and were found satisfactory. The pH is measured after mixing aqueous and oil phases and the visual appearance at the filled product stage.

Identification and assay of active ingredients
The potency test takes place at the stage of vaccine bulk or filled product. The assay was described in sufficient details and was satisfactory validated. Release requirement was established (titre ≥ 6.8 IU/ml).
Overall it was concluded that the final product is formulated on the basis of a validated in-process test for antigen activity. The criteria should ensure that any batch that is released has a potency value consistent with a standard formulated batch, as defined by the limited amount of data currently available. The Applicant has furthermore agreed to review the pass level when data on ten full-scale batches are available.

Setting of the End of Shelf life Specification
The stability data provided show that there is no indication of decline in potency over 21 months of storage. Therefore in order to ensure that product remains fully potent, the end of shelf life for Netvax was set at 6.8IU/ml.
Identification and assay of adjuvants
The methods used to control the content of excipients were described adequately and were satisfactorily validated.

Safety tests
A Target species safety test at the first filled lot from a bulk batch was described in sufficient details. Their serum is tested for the presence of anti-C. perfringens alpha toxin antibodies using an ELISA assay that detects antibodies against C. perfringens alpha toxin. The Applicant proposed to perform this screening for the first 5 batch release safety tests. If all tests are negative or birds have consistently low levels of antibodies, testing will cease and the requirement removed from the SOP.

Sterility and purity tests
Sterility tests at the stage of direct inoculation (Aqueous phase after mixing) and at the final containers are performed and were described in detail. They were found to be in accordance with the relevant Ph. Eur. requirements and were both adequately validated.

Inactivation
Complete inactivation and absence of toxicity were demonstrated by in-process tests and confirmed by the final product sterility test and the in-vivo target species safety test.

Batch to batch consistency
Batch release documentation for three consecutive production batches of Netvax was provided. All of the batches met the required specifications.

STABILITY

Stability of the bulk antigen
A 12 month shelf life for the antigen was assigned.

Stability of the finished product
A stability study was set up using three batches of vaccine with the intention of storage for up to 39 months. An interim report was included. To date, the results cover storage of 21 months. Throughout the above mentioned periods of storage, all three batches met the required specifications for visual appearance, pH, free formaldehyde and thiomersal content.
It was concluded that 2 TCP represents a minimum potency of the vaccine. The target level for blending was at 3 TCP for commercial release and therefore the release specification has been set using 3 TCP batches. Therefore 3 TCP batches were considered by the Applicant as appropriate for demonstrating stability.
On the basis of the data presented a provisional shelf life of 18 months was accepted by the CVMP. The Applicant agreed to carry out further stability studies using batches of vaccine manufactured using toxoid batches that have been stored at 5 ± 3 °C for 12 months prior to blending.

OVERALL CONCLUSION ON QUALITY

The data provided within Part II were generally satisfactory and current guidelines were taken into account. Formulation development was well described and the product composition justified. The starting materials used to produce the vaccine were generally well defined and of satisfactory quality. Details of the manufacturing process and process validation were found satisfactory and the batch analysis data demonstrated consistency of manufacture of the finished product. Based on the stability data provided, a shelf-life of 18 months for the finished product was justified. The TSE risk for this product can be regarded as negligible.
3. SAFETY ASSESSMENT

The safety of Netvax was investigated in two GLP compliant laboratory studies, a non-GCP compliant field trial and a GCP compliant field trial. In the laboratory studies, vaccine formulations containing 4 TCP/dose were used, a higher content than the final formulation agreed for commercial batches, at 3 TCP/dose.

A. SAFETY ASSESSMENT

LABORATORY TESTS

Reports of two laboratory safety studies were presented. Both were carried out in accordance with the principles of GLP.

- The first of these (see below) was designed to establish the safety of a single dose, an overdose and repeated administration of a single dose of vaccine to chickens younger than the minimum recommended age for vaccination.
- The second study was designed to investigate the safety of the vaccine for reproductive birds

Safety of a single dose, an overdose and repeated administration of a single dose study

Six-week-old female broiler breeder chickens were used in the study. A batch that contained 4 TCP units of toxoid per dose and formulated with Drakol 6VR as adjuvant was used and was equivalent to a standard batch that contained the maximum antigen concentration per dose and was therefore acceptable for use in the safety study.

Birds were allocated to treatment groups as follows:

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Single dose</td>
</tr>
<tr>
<td>3</td>
<td>Single dose (controls)</td>
</tr>
<tr>
<td>4</td>
<td>Overdose</td>
</tr>
<tr>
<td>6</td>
<td>Overdose (controls)</td>
</tr>
<tr>
<td>7</td>
<td>Repeat dose</td>
</tr>
<tr>
<td>9</td>
<td>Repeat dose (controls)</td>
</tr>
</tbody>
</table>

Controls (groups 2, 5, 8) were vaccinated with saline and the other groups with a Netvax batch containing an antigen level of 4 TCP.

Vaccines were administered according to the following scheme:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Treatment group nos.</th>
<th>Volume (ml) and time of administration (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose</td>
<td>1, 3</td>
<td>0.5 (L) 0.5 (R) 0.5 (L)</td>
</tr>
<tr>
<td>Overdose</td>
<td>4, 6</td>
<td>1.0 (L)</td>
</tr>
<tr>
<td>Repeat dose</td>
<td>7, 9</td>
<td>0.5 (L) 0.5 (R) 0.5 (L)</td>
</tr>
</tbody>
</table>

Follow up

Birds were monitored for the following parameters: a) Local reactions, b) Systemic observations, c) Weight gain, d) Serology, e) Necropsy

Results

Serology: With the exception of one bird in the single dose group, all birds vaccinated with the vaccine were seronegative for antibodies to C. perfringens alpha toxin. Serology seven days later showed no significant increase in antibody titre for any bird indicating that there was no active infection.

Local injection site reactions: No specific local injection site reactions were observed in any bird.

Single dose group: Following single dose treatment, swelling of the breast muscle was first observed 11 days after initial vaccination in 13.1% birds, increasing to 46.7% by 12-18 days and resolved in all birds by day 30. Following the second vaccination on day 56, generalised swelling of the breast muscle was first observed five days later in one bird (this persisted until the end of the study on day 75); swelling was observed in only one other bird on one day only.
Overdose group: Generalised swelling of the breast muscle was first observed ten days after the initial (double) dose in 22.2% birds, increasing to a maximum of 12/18 (66.7%) on day 21 and resolving in all birds by day 28. Following the second vaccination on day 56 slight swelling of the breast muscle was observed seven days later in one bird and slight softness of the muscle tissue in another bird after 14 days.

Repeated dose group: Generalised swelling of the breast muscle was first observed nine days after the initial dose in 11.1% birds, varying between 11.1 and 66.7% between days 10 and 21. Following revaccination on day 21 the percentage of birds with swollen breast muscle ranged between 11.1 and 44.4%. Over time the swelling reduced in size but was still detectable in three birds at the time of the third vaccination on day 56. The number of birds with swellings increased after the third vaccination from 30.76% to 46.15 % of birds and these were still resolving at the end of the study on day 82.

Post-mortem examination: In most vaccinated birds the muscle surface was typically pale with some striations. In many cases serous fluid and/or caseous material was present between the muscle layers of the breast which appeared to be resolving leading to the formation of nodular and fibrous material between these muscle layers.

Histopathology: For birds treated with an overdose, multi-focal areas of necrosis were present in the breast muscle 21 days after the first vaccination along with granulomas containing macrophages and multinucleated giant cells. The findings were regarded as typical of a vaccine reaction.

No systemic reactions in any treatment group and no vaccine related mortalities were observed.

Weight gains: There were no statistically significant differences in mean weight gain between any of the treatment groups and their. Local reactions may increase slightly if twice the recommended dose is administered.

Conclusions: Overall the study provided a satisfactory demonstration of the safety of the vaccine formulated to contain the maximum amount of antigen specified per dose to broiler breeder chickens younger than the minimum recommended age. Therefore the following conclusions can be supported:

Safety of the administration of one dose
There were no systemic reactions to the vaccine and the main local injection site reaction was swelling of the breast muscle tissue. The warning proposed for section 4.6 of the SPC was revised to adequately describe the reactions seen and in particular the fact that swelling may persist for at least 35 days after the 2nd vaccination.

Safety of an administration of an overdose
Reactions were in general similar to those seen following administration of a single dose. On the basis of the results the Applicant has concluded that reactions may increase slightly if an overdose is administered. The warning proposed for section 4.6 of the SPC was revised to reflect that local reactions may increase slightly if twice the recommended dose is administered.

Safety of the repeated administration of one dose
Repeated administration of a single dose did not result in reactions more severe than those seen following administration of a single dose.

Examination of reproductive performance study
Broiler breeder chickens of approximately 22 weeks were included and divided in two groups (Group1 and Group 2. They had been already vaccinated with the normal range of commercial vaccines. None of these were likely to have influenced the results of this safety study. A Batch was blended to contain 4 TCP units of toxoid/dose. The vaccination scheme was as follows: Group 1 (Vaccinates) was vaccinated intramuscular (i.m.) with 0.5ml of the vaccine at the beginning of the study and again 4 weeks later. At the same time intervals Group 2 i.m. saline and were kept as controls.

Follow up:
The following parameters were monitored: a) Serology, b) Local systemic reactions c) Local injection sites, d) Weight gain, e) Egg Production and Hatching of Chicks: Twenty five eggs per group, laid before the second vaccination, were incubated and candled to establish fertility prior to the completion of the vaccination regime. Eggs laid during the two weeks immediately following the second vaccination were counted and then discarded. Eggs laid thereafter, were collected twice daily, weighed
and examined for quality. Once 100 eggs from each group were collected, they were placed in two separate, labelled trays of one hatcher, for incubation and candling. Viable eggs were hatched and the number of chicks hatching from each group recorded.

**Results**

**Vaccination phase**

Serology: Some hens from the vaccinated group did not meet the criteria for inclusion in the study as they demonstrated an anamnestic response.

Local and Systemic Reactions: No systemic reactions were noted post vaccination.

Mortalities: There were no vaccine related mortalities

Weight Gain: The average weight of control hens and vaccinated hens was almost identical throughout the study, with both groups remaining slightly under the average target weight throughout the study.

**Egg production phase**

The onset of lay for both groups was 18 days post the first vaccination (V1). The total number of eggs collected prior to the second vaccination (V2) (a 10 day period) was 48 from the control group and 119 from the vaccinate group. From these 25 eggs per groups were selected at random checked for fertility. There were 20 fertile eggs from the control group and 16 from the vaccinate group.

Following V2, the total number of eggs laid was 303 from the control group and 431 from the vaccinate group. Of these, the most recent 100 were selected for hatching. There were no misshapen or obviously abnormal eggs from either group. Hens were 28 to 31 weeks of age during this period.

Weight of eggs: The mean weight of the 100 eggs selected was 57.8g from the control group and 58.1g from the vaccinate group.

Thickness of shells post hatching: The average shell thickness was 0.36mm for eggs from the control group and 0.35mm for eggs from the vaccinate group.

Fertility of eggs: The difference in percentages of chicks hatched from both groups was not statistically significant.

**Chick phase**

Mortalities: The difference in percentage mortalities between the two groups was not statistically significant.

Weight Gain: All surviving chicks remained healthy and displayed no abnormalities. Statistical analysis found that at day 1, week 1, week 2 and week 3, the mean weight of chicks from vaccinated hens was significantly higher than chicks from control hens.

**Conclusions:**

The CVMP concluded that taking into account the results of this study and the Applicant’s clarifications and comments the vaccine is safe for laying and breeding birds.

**Examination of immunological functions**

The inactivated antigens used in the vaccine are known to induce the production of specific antibodies in vaccinated animals. There is no evidence to suggest that this vaccine may have any adverse effect on the immune response of the vaccinated animal.

**Interactions**

No information is available on the effects of the concurrent use of the vaccine with any other. A standard warning is therefore included in section 4.8 of the SPC.

**FIELD STUDIES**

Two reports from field trials were presented in this part of the dossier. One of the two was a proof of principle trial while the other relates to large scale GCP field trials in Germany and Italy. Only the aspects of the studies relating to safety of the vaccine will be discussed here.
• Field efficacy testing of a necrotic enteritis vaccine for poultry

Healthy broiler hens, with no previous history of vaccination against clostridial disease, and their progeny took part in the study. An Experimental batch of the vaccine was used, formulated to contain 2 TCP units of toxoid per dose. The CVMP noted that the vaccine batch contained less than the standard target quantity of antigen per dose but was within the range specified for the product and met all other specifications. It can therefore be accepted that this study provided additional information on the likely safety of Netvax when used in the field. Approximately 38% of the hens were vaccinated and approximately 30% of the hens were similarly injected with 0.9% saline as controls. Hens were vaccinated by intramuscular injection of 0.5 ml at 11 (V1) weeks and 18 (V2) weeks of age. Hens were reared to 21 weeks of age and then transferred to a broiler farm as replacement breeder hens.

Follow up:
Broiler breeder hens: The birds were observed twice during the first 24h following each vaccination and then daily for 14 days for clinical signs of adverse reactions. In addition egg production and viability was also monitored as a measure of bird performance.

Chicks: Weight gain was assessed by weighing of randomly selected chicks from each treatment group over the initial three weeks of life. Body weight was also determined at slaughter. The feed conversion ratios (FCR) were calculated for days 21 and at slaughter (day 56).

Mortality was determined over the initial three weeks of life and at slaughter. Daily observations were made over the initial three weeks of life for clinical signs of necrotic enteritis including depression, ruffled feathers, inappetance, closed eyes, immobility and dark coloured diarrhoea.

Ten clinically normal chicks from each house were selected on days 1, 7, 14 and 21, and after being euthanized were submitted to post mortem examination for the presence of intestinal lesions. Where lesions typical of dysbacteriosis were observed, the clinical scoring system of JF Prescott was followed. Blood samples were collected from each of fifty randomly selected birds from each treatment group at the time of each vaccination and at 21, 25, 30, 40, 50 and 58 weeks of age.

At weeks 25, 40 and 58, eggs were collected from the broiler breeder hens in each of the treatment groups and hatched and placed according to normal practice. Chicks from each treatment group were observed daily for clinical signs of necrotic enteritis for a period of 21 days. Blood samples were collected from fifty chicks in each treatment group on days 1, 3, 7, 14 and 21. Specific antibody against C. perfringens type A alpha toxin in IgY (from egg yolk), hen sera and chick sera were quantified by direct ELISA.

Results:
The vaccine was well tolerated and no adverse systemic reactions were observed under field conditions. Some swelling was observed at the injection site, particularly after the second vaccination. These local reactions were found to persist for at least 14 days in some birds. There was no detectable effect on reproductive performance in the field.

For broiler chickens hatched from eggs produced by the breeder hens at different ages, there was no discernable overall trend towards improved or reduced performance in vaccinated birds. Improved performance (body weight, FCR and mortality) was however observed in birds from vaccinated hens for the 25 week hatch.

Conclusions:
The CVMP concluded that vaccination did not have an adverse effect on mortality or weight gain compared to the controls.

• Field safety and efficacy testing of a necrotic enteritis vaccine for poultry

Broiler breeder hens and their progeny were included at two sites, one in Germany (01) and one in Italy (02). In each site birds were divided in two groups and one group was vaccinated and the other remained as controls. Breeder pullets were vaccinated with 0.5 ml i.m. at 11 weeks of age and a second dose was given at 18 weeks old (in site 2) or 19 weeks old (in site 1).

A batch that contained 3 TCP was used; this was equivalent to a standard batch of vaccine containing 3 TCP units of toxoid per dose.
Follow up:
Hen Vaccination Phase
The vaccinated birds were observed for adverse reactions twice during the first 24 hours following each vaccination and then daily for 14 days. Randomly selected pullets were examined for three weeks for the presence of injection site reactions.
Serum was collected from randomly selected breeder hens from each treatment group before each vaccination. Eggs were also collected in a single day at the same time, from each treatment group for IgY extraction.

Egg production Phase
Egg production was monitored as a measure of bird performance during the whole study period.
On weeks of bird placement, eggs were separately collected from each treatment group and separately incubated to determine the hatchability and to monitor the offspring.

Chick Phase
Following hatching, chicks were monitored daily. Chicks were weighed on a weekly basis for the first 8 weeks. Serum samples were collected from randomly selected chicks from each treatment group on Day 7. For the first four weeks, all chicks were observed daily for signs of necrotic enteritis. In the event of a clinical disease outbreak, birds were euthanized and examined for the presence of necrotic enteritis specific lesions. In addition, mortality and feed consumption were recorded. Feed conversion ratio and carcass quality were recorded at slaughter.

Results:
Hen Vaccination Phase
No systemic reactions or adverse clinical signs were observed in pullets/hens at either site. All pens were scored as normal during weekly pen observations. No adverse events were observed in broiler chicks. No deaths related to the vaccine occurred. No *Clostridium perfringens* associated gross gut lesions were observed and therefore no bacteriological analysis was conducted.

Injection site reactions were observed in a total of 4.2% birds from the vaccinated group at site 1.6% of vaccinates observed during the 3 weeks after V1 showed injection site reactions, 1.3% in the 3 weeks after V2, and none of the vaccinated birds observed at end of study had injection site reactions. Some haematomas were observed in the week following vaccination. These resolved to be replaced with generalised swelling and discolouration. The maximum size of the observed swelling was 30 x 20 mm. These reactions were not considered severe by the investigator and none were of welfare concern. At the week 35 observation time point, 2 fibrosis reactions were observed in the vaccine group. At the end of the study (week 42), none of these reactions were persistent. The incidence of reactions was reduced following the second vaccination.
At site 02, 0.7% of vaccinates observed during 3 weeks after V1 showed injection site reactions, none during the 3 weeks after V2, and 8.0% of birds observed at the end of study (week 32) had injection site reactions. From all sampling time points through the end of the study, 0.9% of vaccinate birds showed signs of injection site reactions. The control group was mistakenly not observed for injection site reactions after V1. Over all sampling time points, 0.9% of control birds showed signs of injection site reactions. At the end of the study (week 32) over the birds observed, 0.7% were reported with signs of tissue fibrosis. Some birds exhibited reaction on both sides of the neck. The maximum size of these reactions was 3 x 12 mm. They were not of welfare concern. It is possible that, due to their location in the neck, these reactions were not due to vaccination with Netvax.

Egg production Phase
Egg production was comparable between vaccinates and controls. At both sites, hatchability rates were similar for vaccinates and controls. Following statistical analysis it was shown that there were no statistical effects of vaccination on the number of eggs produced or the percentage of hatchability of the eggs.

Chick Phase
• Mortality: Over the whole study period the mortality rate ranged from 1.39 to 1.93% in the control group and 0.77 to 2.38% in the vaccine group at site 01 and 2.76 to 3.91% in the control group and
2.76 to 3.31% in the vaccine group at site 02. These differences in mortality percentage were statistically significant at site 01 for hatch week 45 and at site 02 for hatch week 27.

- Weight gain in chicks: Differences in mean weights between vaccine and control groups at site 01 and site 02 were not statistically significant.
- Feed consumption in chicks: At site 01 the feed conversion ratio was slightly higher in the vaccine group for both batches of chicken followed. Ratios were comparable between the vaccine and the control groups at site 02. The ratios achieved were within the standards of the two companies.

Observations at slaughter: For both sites the number of rejected birds or yields were similar for the vaccinated and control groups and figures were comparable to the usual figures observed with birds from the companies.

Conclusions:
The CVMP concluded that this study adequately demonstrated the safety of this vaccine when used in the field. Reactions and performance observations were similar to those in the laboratory safety studies.

ENVIRONMENTAL RISK ASSESSMENT

A satisfactory Phase I assessment of risk for this inactivated vaccine, in accordance with EMEA/CVMP/074/95, was performed. The final product contains no components which may exert a toxic effect and there are no pharmacologically active components included in this vaccine. On the basis of the phase I assessment where it was concluded that the environmental risks presented by the use of Netvax in the field are negligible, a phase II assessment was not considered necessary. No special precautions are considered necessary for disposal of waste material; empty containers and unused product may be disposed of by standard procedures.

USER SAFETY

The presence of mineral oil in the adjuvant could present a risk to the user if it is accidentally self-injected. The standard recommended mineral oil safety warning has been included in section 4.5 of the SPC.

B. RESIDUE ASSESSMENT

MRL

Sodium chloride, formaldehyde, thiomersal, EDTA, light mineral oil, sorbitan oleate, polysorbate 80 and benzyl alcohol are included in Annex II of Council Regulation (EEC) No. 2377/90.

Triethanolamine, which is included as an excipient in the final product, was not included into Annex I, II or III of Council Regulation (EEC) No. 2377/90. The Applicant therefore presented a justification that this material is not pharmacologically active at the concentration used in the final product and therefore acceptable for use in a veterinary medicinal product. Reference was made to advice in the CVMP’s “Position paper on the definition of substances capable of pharmacological action in the context of Council Directive 2001/82/EC as amended, with particular reference to excipients and manufacturing materials” (EMEA/CVMP/072/97-Rev-1). A total of five studies (four in rats and one in dogs) were presented. In these studies, the dose of triethanolamine used was 0.25 mg/kg and 0.4 mg/kg bodyweight while the dose of triethanolamine received by a 1-2 kg chicken over the vaccination course was 0.35 to 0.76 µg/kg bodyweight.

On the basis of the data submitted the CVMP considered that the triethanolamine was not pharmacologically active when used as excipient at doses up to 0.25 mg/kg bw and therefore at these doses does not fall within the scope of Council Regulation 2377/90.

WITHDRAWAL PERIOD

The withdrawal period is zero days.
OVERALL CONCLUSIONS ON SAFETY

The safety of Netvax was investigated in a series of laboratory studies and field trials. The safety of single dose, overdose and a repeated single dose was established in chickens of 6 weeks of age. This represents a younger age and therefore more sensitive target animal than the recommended age for vaccination of 10 to 14 weeks of age. No systemic reactions were observed. The only local injection site reaction observed was generalised swelling of the breast which resolved within 30 days. These are adequately described in section 4.6 of the draft SPC. Following the second vaccination the incidence of swelling was less. Vaccination had no effect on weight gain. In order to assess the effect of Netvax on reproductive performance, hens of 23 weeks of age, on the point of lay, were vaccinated with a high potency vaccine. Although this is beyond the recommended age for vaccination, it probably represents a more sensitive category for breeding birds, hens at the point of lay. Vaccination of hens at this point in their reproductive cycle had no negative effect on hen health or weight; egg morphology, weight or hatchability; or chick health or weight.

The limited nature of the reactions seen in the laboratory studies has been supported by the results of field trials. The Applicant has also satisfactorily demonstrated that triethanolamine, which is included in the vaccine as an excipient but is not listed in the annexes to Council Regulation (EEC) No. 2377/90, is not pharmacologically active at the concentration included in the vaccine.

As the suitability of the batches of vaccine was confirmed, it can be concluded that the vaccine is safe for chickens of the youngest recommended age and also for breeding birds.
4. EFFICACY ASSESSMENT

INTRODUCTION

Necrotic diseases in domestic chickens are associated with *Clostridium perfringens* type A. It is common in the gut of chicks and is found in the general poultry production environment. It has been identified as occurring as early as 2 weeks of age. Necrotic enteritis is a multi-factorial disease characterised by reduced performance of broiler birds and increase in mortality during the production cycle. Outbreaks can occur as early as 7 days of age but are most common in birds of 2-3 weeks of age. Necrotic enteritis can be clinical (acute and mild), or sub clinical. In the acute form birds can die with external diseases signs. Mortality can be up to 50%. In the sub clinical form signs include huddled feathers, depression, diarrhoea and decreased food consumption. Lesions are found in the small intestines and sometimes in the liver and kidney and caeca of birds suffering from NE.

The rationale behind vaccination against *Clostridium perfringens* type A alpha toxin lies with the knowledge that antibodies against alpha toxin appear to have a role in protection against NE. Heier noted that chicks with high levels of MDA against alpha toxin had lower mortality rates during production. The intended schedule is for a primary dose of 0.5ml to be given at 10-14 weeks of age followed by a booster vaccination of 0.5ml 4-10 weeks after the primary vaccination. The route is intramuscular in the breast muscle.

LABORATORY TRIALS

Nine laboratory studies were submitted in order to support the efficacy of the vaccine. Four of them involved the establishment of a challenge model, another four related to the onset of protection and duration of immunity and one aimed at establishing the appropriate window of vaccination. Three of the studies investigating the onset of protection also investigated the passive protection of the chicks. The efficacy of this vaccine relies on passive transfer of immunity from hen to chick via egg IgY and in all key efficacy studies, levels of specific egg IgY were by HIA. This has enabled to establish a minimum antibody level shown to be protective. In turn, it was recognised that the amount of IgY transferred to the egg and subsequently to the offspring is directly related to the circulating levels of IgY in the hen. Therefore it was established that there is a correlation between specific egg IgY quantified by HIA and protection from Necrotic Enteritis. The CVMP concluded that there is a general association between the results of the two tests.

Establishment of a Challenge Model

Due to the multi factorial nature on necrotic enteritis the Applicant had to examine a combination of contributing factors to establish a laboratory model. Four specific studies were conducted evaluating combinations of parameters. None of them formed the definitive challenge model although aspects of some of the protocols were included in the final model. Birds in these studies were from 14– 20 days at challenge. The model chosen by the Applicant was based on stressing chickens by delivery of a high protein diet followed by a *C. perfringens* challenge at 3 weeks of age. The CVMP considered that although the challenge model was not representing a natural challenge model, it did demonstrate the need to have *Clostridium perfringens* to initiate disease and could be said to be a more severe model than field disease.

Onset of protection and Duration of Immunity.

The Applicant presented four laboratory studies to support the onset of protection and duration of immunity and is summarised below. Studies 2 and 3 also aimed to demonstrate the passive protection in broiler chicks.
• **Study 1**

**Study design**

SPF chicks, of 10 weeks of age at the start of the study, were divided into groups. Each group was housed in a separate hut. The birds were monitored for any adverse reactions or health sign prior to vaccination. They were vaccinated intramuscularly with 0.5ml of vaccine at 10 weeks of age and a booster dose given at 4 weeks following initial vaccination. Only group 1 was vaccinated. The final blend contained 2TCP per dose and thiomersal and gentamicin.

**Follow up:**

Blood samples were collected at various time points until 65 weeks of age. A total of 5 eggs were collected from each group at week 30 through to week 55 of the study. The yolks of each group were pooled and processed to determine antibody titre.

**Results**

Antibody titre as measured by HIA showed that control birds had low/negative antibody titre over the duration of the study (mean 3 or less). The geometric mean of the HIA titre in vaccinations showed a significant titre. An HIA titre of >64 was considered to be seroconversion.

The CVMP concluded that this study showed that vaccination of hens at 10 weeks and 14 weeks of age, using a batch formulated at minimum antigen input, 2TCP, induced antibody formation which persisted across the laying period. It also showed that antibodies can be detected in the yolk of eggs from vaccination chickens.

Following presentation of further data there seemed to be some variation between the egg and hen antibody titres, especially at lower titres, but a general association can be confirmed.

• **Study 2**

**Study design**

This study involved a challenge of progeny from hens vaccinated with the test vaccine. Progeny from eggs at 32 and 54 weeks of age of laying hens were used. Pullets and roosters were used at 10 weeks of age. They were divided into 2 groups. All pullets in group 1 were vaccinated intramuscularly with 0.5ml vaccine at 10 weeks of age. A booster was given at 23 weeks of age. Group 2 was not vaccinated and remained as a control group. The vaccine used was formulated at 2 TCP/dose. The product was tested for sterility and potency only. Examination of the data suggests that the potency was probably not the minimum specified for this test. However, the lack of any results using the now adopted HIA assay makes any further conclusions impossible.

Blood samples were taken at the week before the start of the study, week 25 and week 45. Eggs were collected from each group at weeks 22 and 44. The yolk from 5 eggs from each group was processed and pooled and tested by HIA. On week 22 (hens 32 weeks of age) 72 eggs from vaccinates and 70 eggs from controls were set for hatching. Chicks were hatched and brooded until 18 days of age. Challenges were performed in birds hatched from hens at 32 weeks of age and again in birds hatched from hens at 54 weeks of age. Chicks were divided into 3 groups. Group 1 originated from vaccinated hens, group 2 and 3 from controls. Only groups 1 and 2 were challenged. The challenge was performed at an age of approximately 3 weeks. Back titrations were performed to confirm the challenge dose.

**Follow up:**

Chicks were observed daily for illness or death until the end of the study. Post mortems were conducted at the end of the study. Lesions in the intestines and duodenum were scored according to a scale of 0-4 as described early in this report.

The interval between the 2 doses of vaccine is outside the recommendation but can be regarded as a worst case scenario.
Results:

Hens: Antibody titres in serum results were presented. Hens vaccinated at 10 and 23 weeks of age had significant serum titres of antibodies against Clostridium perfringens at 35 weeks of age. At 55 weeks of age, 84.5% of vaccinated birds still had titres above the level considered to be representative of seroconversion. The geometric mean titres in 35 and 55 week old vaccinates were significantly higher than the controls, p<0.0001. Hens with a HIA titre above 64 were considered to have seroconverted. The geometric mean HIA antibody titre in pooled egg yolk taken from vaccines at 32 and 54 weeks of age was significantly higher than controls, p<0.0001.

Efficacy in progeny chicks:

- 32 weeks of age derived chicks
A total of 72 eggs were set for hatching, however, only 11% healthy chicks were hatched from this group (vaccinated hens). The Applicant speculated that a large number of infertile eggs and or malfunctioning of the egg incubator could have been the causes of this. None of the progeny chicks from vaccinated hens developed NE lesions, whereas 76% of the chicks from control hens did. The challenge was considered severe enough as it resulted in the deaths of 2 chicks from the control group. Mean lesion score in non vaccinated challenged controls was 1.7 (ranging from 0-4) and in vaccines 0. This was shown to be statistically significantly different.

- 54 weeks of age derived chicks
Only 25% of healthy chicks were hatched from the total amount of eggs. The reasons speculated for the poor hatch were as previously described (the way eggs were packed). None of the progeny chicks from vaccinates developed lesions whereas the 37% of chicks from unvaccinated hens did. The incidence of lesions in the chicks from vaccinates was significantly lower (p=0.0183) compared to controls. Progeny from vaccinated hens had a lesion score of 0 and the progeny from the control group had a mean score of 0.7 (0-3). This was statistically significant.

Conclusions:
This study showed that hens vaccinated at 10 and 23 weeks of age with a batch of minimum antigen input had serum titres of antibody at 32 and 54 weeks of age against Clostridium perfringens. Eggs produced at these ages also had significant antibody titres but these dropped over time. Chicks produced from vaccinated hens at 32 and 54 weeks of age and challenged at 18-20 days of age were protected against the development of lesions, however, the challenge at 54 weeks of age was weak and at both time points the numbers of chicks challenged were very low and therefore not statistically significant.

- Study 3
Study design:
Female breeders and roosters were vaccinated at 10 weeks and 20 weeks of age (booster). Similar amount of birds were used as vaccinates and controls. The antigen input of the batch was 2 TCP. Birds were vaccinated with 0.5ml. Blood samples were collected from 20 hens in each group at 25 weeks of age and tested in the ELISA for alpha toxin. Eggs were collected from each group at 26 weeks of age (week 16 of the study). The eggs were set for hatching in Elkhorn. The yolk from randomly selected eggs from each group was processed for testing in the ELISA and HIA tests. Progeny chicks were commingled in 2 huts. Groups 1 and 2 included birds originating from vaccinated and control hens respectively. They were challenged with Clostridium perfringens at approximately 3 weeks of age. The third group included birds that were not challenged.

Follow up:
Chicks were monitored daily for illness or death until the end of the study which was at 24 days of age. Post mortems were conducted at the end of the study and NE lesions scored from 1-5.

Results:
A total of 174 chicks were hatched. Eighty nine were used in the study and of the remaining ones, 16 were bled the rest entered into a pool for further blood collection. The percentage hatchability of
fertile eggs from vaccinates was 88% and from controls 92. The criteria for a valid challenge were not met in hut 51 as only 47% of birds in the control group developed lesions. This group was not entered into statistical analysis.

In hut 52, 68% of control birds developed NE lesions compared to 24% of vaccinates. No NE was observed in unchallenged controls although it is noted that of the 5 controls 2 died of causes specified not to be due to NE. Mortality was recorded in control birds. Differences between scores for vaccinate and controls were statistically significant with vaccinates demonstrating significant protection.

Antibody levels in vaccinated hens were higher than controls.

From the results it was evident that vaccination of breeder hens resulted in the transmission of antibodies to eggs, which has persisted to week 26 of life of the breeder hen.

It was seen that chicks had antibodies at 1 day of age which persisted in a proportion of chicks until day 10 (taking > 40 as the baseline for seroconversion). At day 10 half of the chicks still had antibody levels above 40. Hens vaccinated at 10 weeks of age and boosted at 20 weeks of age with a batch of vaccine at minimum antigen input and possibly minimum potency were seen to have significant titres at 25 weeks of age, 100% >40. However, the control group had 70% of birds with a titre > 40. Eggs produced by vaccinated birds at week 26 also had significant titres whereas eggs from non vaccinated hens did not. Chicks born from vaccinated hens at week 26 of age and challenged at approximately 3 weeks of age were protected against mortality (0%) and had a reduced incidence of lesions compared to control chicks born from non vaccinated hens. Chicks from vaccinated mothers had antibody titres over the first 10 days of life. Antibody titres by ELISA in progeny at the days of challenge, 19-21 days were below the level of seroconversion (>40).

Conclusions:
This study offered evidence of a reduction of lesions rather than prevention of lesions and this is in progeny from hens aged 26 weeks of age. The analysis of the efficacy data provided adequate support for a claim that the vaccine reduces mortality due to necrotic enteritis.

Study 4
The Applicant also conducted this study to assess the transfer of passive protection from individual bird to individual eggs. Birds were vaccinated under field conditions and eggs transferred to the laboratory for hatching and chick challenge.

The pullets in each group (vaccinates, group 1 and controls group 2) were kept in separate flocks. Broiler breeders were vaccinated at 10 and 17 weeks of age with a batch of vaccine, formulated at 2.5 TCP. Randomly selected hens were bled at 39 weeks of age. Eggs were collected on a single day from hens aged 40 weeks. The eggs were used for hatching and challenge of chicks. Five eggs from each group were tested for specific antibodies 79.3% of eggs from the vaccinated group hatched and 71% of the control group. Eggs were separated by treatment group until hatched and hatched chicks were then separated by treatment group and housed separately. Chicks were divided into 3 groups. Group 1 consisted of progeny from vaccinated parents. Group 2 consisted of progeny from the control non vaccinated parents. Group 3 consisted of chicks originating from non vaccinated parents and subsequently acted as a control group for groups 1 and 2. Progeny chicks were challenged at approximately 3 weeks of age with Clostridium Perfringens.

Follow up
Chicks were observed daily following challenge for illness or death until the end of the study (3 days after the last day of challenge). Birds that died after challenge and also all birds euthanased at the end of the study were subjected to post mortem and NE lesions scored in the intestine and duodenum. The scoring system was on a scale of 0-4.

The challenge method and scoring system met the standard model described by the Applicant in the dossier.

Results:
At least 60% of the challenged progeny chicks from control hens showed NE lesions following challenge and the progeny from vaccinated hens demonstrated significantly lower NE lesions.
compared to progeny from control hens. Control hens remained seronegative throughout the study. This means that the criteria for a valid test were met.

The hatching rate was 79% in vaccinated chickens and 71% in non vaccinated chickens. The mortality rate in vaccinated chickens was 3% and 14% in the control group. All these birds showed signs of necrotic lesions (Turkish towel appearance). The incidence of NE lesions in the control group was statistically significantly higher (p=0.0021) than the control group. The lesion score in the vaccine group was significantly lower (p=0.0494, 2 sided) than the lesion score in the control group by Wilcoxon Rank sum exact test. The Mitigated Fraction method has been used to determine the efficacy of the vaccine. The statistical relevance of this method was justified. Mean antibody titres in the serum of vaccinated hens at 39 weeks of age were 194 as measured by HIA. In non vaccinated chickens it was <2. The HIA titre in egg yolk of eggs collected from vaccinated hens at 40 weeks of age was 256 and non vaccinated hens at 40 weeks of age <2.

Conclusion:
This study also supported the claims for a reduction in the incidence of necrotic lesions and mortality in chicks born from hens vaccinated in accordance with the proposed vaccination schedule

**Intended schedule of vaccination**
The Applicant provided a study to support the different time windows allowed by the proposed schedule of vaccination. The intended schedule is for a primary dose of 0.5ml to be given at 10-14 weeks of age followed by a booster vaccination of 0.5ml 4-10 weeks after the primary vaccination. The route is intramuscular. The study is described below:

- **Study 5**

  This study was designed to compare the antibody response of the hen at 27 weeks of age (considered to be the onset of lay) following vaccination at the extremes of the proposed vaccination schedule. Nine week old, female, Breeding stock were used. These were housed in floor pens and fed a restricted diet until 21 weeks of age after which the feed was changed to poultry breeder feed. They were divided into groups as below:

<table>
<thead>
<tr>
<th>Group</th>
<th>Age at vaccination</th>
<th>Time of bleed (weeks of age)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>V1</td>
<td>V2</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

V1: First vaccination, V2: Second vaccination

Birds were bled as described in the table above. Birds were vaccinated at the ages stated in the table above with an experimental batch which contained 2TCP/dose.

**Results:**
The Applicant presented a summary table of results. It was confirmed that only 12.5% of the control birds had high titres (up to 320) at 23 and 27 weeks, although 6% had a high titre (160) at 20 weeks and 12.5% seroconverted with titres of 40 by 20 weeks and in one case 80 at 27 weeks. There were no overall significant differences in the mean antibody responses among vaccine groups at 23 or 27 weeks of age (p=0.1877 and p=0.3777 respectively). The differences between groups 1 and 4 were statistically significant at 23 weeks of age.

**Conclusions:**
The results of the study were found acceptable. On the basis of the above data the statement in section 4.2 of the SPC has been revised accordingly.
FIELD TRIALS

The Applicant conducted small scale non GCP trials in France and Italy with different formulations of the vaccine. The Applicant also conducted full scale GCP trials in Germany and Italy. However due to some problems in serological measurements and no substantial evidence of true challenge these studies were only regarded as supportive to the claims and not pivotal.

- **Small scale field trials**
  
  **Study design:**
  Birds were allocated either to a vaccinated group or a control group. Each group was housed separately. The vaccinated group was 3 days younger than the control group. Vaccinates received 0.5ml of vaccine by IM injection at 11 weeks and 18 weeks of age using a batch with 2TCP antigen input.

  **Follow up:**
  Following each vaccination birds were observed twice during the first day and then daily for 14 days. At week 21 birds were transferred to a breeder site as replacement breeder hens. Serum was collected from randomly selected hens from each treatment group at each vaccination and at weeks 21, 25, 30, 40, 50, and 58. These were tested for HIA and ELISA titres. Serum was tested in 5 pools of 10 samples.

  Egg production was monitored as a measure of bird performance. Chicks were hatched on weeks 25, 40 and 58 (+/- 4 weeks). These were grown on and sent to broiler farms. Serum from chicks from each group was collected. For the 1st 8 weeks all chicks were observed daily for signs of NE. If disease was detected chicks were euthanased and examined for the presence of specific intestinal lesions. Lesions were scored on a 1-4 scale described. Intestinal samples were collected from the upper and mid gut and stored frozen until it was possible to enumerate Clostridia. The presence of wet litter syndrome was investigated as it is caused by fluid faeces and considered to be clinical signs of NE typically seen in the field.

  **Results:**
  The signs of NE were seen in both control and vaccinates from the 25 week hatch but not in vaccinates from the 40 week hatch and some signs in the 58 week hatch of both vaccinates and controls. Faecal litter counts from all house showed similar patterns and indicated similar levels of challenge for all groups.

  The pattern of counts from intestinal contents mirrored the results of faecal counts. Counts increased to reach 10^3-10^4 counts/gram by the 21st day of production cycle. Samples from the mid gut gave slightly higher counts. Lesions scores in chicks were presented and some slight improvement in scoring of the vaccinates was observed but not significant.

  **Hens**
  Vaccinate hens had statistically significantly higher titres than control birds from week 18 onwards (p, 0.00179). Peak antibody responses were obtained by 3 weeks after V2 and declined to week 58. Titres in control birds were high at the beginning of the study but dropped at 25 weeks of age.

  **Egg extract pools**
  The antibody levels in eggs from vaccinates were statistically higher than in control birds eggs (p=<0.0476). The titres in eggs from vaccinated hens were statistically significantly higher than control hens eggs at each time point, p<0.0235.

  **Chicks**
  Titres of chicks from eggs laid from 25 week vaccinated hens had the highest mean titres and remained statistically higher than control chicks at days 1, 3 and 7.

  Titres of chicks from eggs laid from 40 week vaccinated hens remained statistically higher than control chicks at days 1and 3. The Applicant considered these results to be anomalous given the higher control values compared to vaccinates.

  Titres of chicks from eggs laid at 58 weeks from vaccinated hens remained statistically higher than control chicks at days 1, 3 and 7.
From the data presented it was seen that the titres drop off quickly and in chicks born from hens at 58 weeks the drop off is quicker.

**Conclusions:**
This study offered information about the efficacy of the vaccine in the field in a supportive context. It also offers some information about the HIA and ELISA titres correlated or otherwise to protection.

- **Full scale field trials**
This study was conducted in Germany and Italy. Site 1 consisted of 3 farms in Germany covering the different stages in chicken production. Birds on this site included: Pullets/hens, Chicken broilers of hatch week 30, 35 and 42.
Site 2 consisted of 4 farms in Italy covering similar periods as site1. Birds on this site included: Pullets, hens, Chicken broilers of hatch week 27, 35 and 32.

**Study design:**
In each site birds were divided in controls and vaccinates. Vaccinates were vaccinated at 11 and 19 weeks in site 1 and at 11 and 18 weeks at site 2. The controls group were untreated. Hens were vaccinated with a standard field dose of 3TCP, containing the target quantity of antigen intended for commercial batches.

**Follow up**
Serum was collected from randomly selected hens from each group before vaccination and at week 25, 30, 35 and 42. Eggs were also collected in a single day at the same time as bleeds from each treatment group. Samples from eggs were tested for specific antibodies by ELISA and HIA. Chicks were hatched from eggs and monitored daily for signs of NE. In the event of specific diseases they were euthanased and examined for specific lesions. Blood samples were collected from the same number of randomly selected chicks, as hens. For the first 4 weeks chicks were observed daily for specific signs of disease. In the event of a disease outbreak chicks were euthanased and examined for signs of NE lesions. Infected gut or hepatic tissues were cultured. Weekly observation of the flocks was conducted. Serum samples were specifically analysed by ELISA.

**Results:**
Hens were considered to have seroconverted if antibody titres were >40 by ELISA. Seroconversion was >80% at V2 at 25 weeks, 100% at 27 weeks, 100% at 30 weeks, 91% at 32 weeks, 62.5% at 35 weeks and 92% at 45 weeks. Seroconversion ratios for control birds were less than 12% at all points at site 01 and less than 20% at site 02.

The highest levels of antibodies in the control groups were observed in the late phase of the study at 35 and 45 weeks of age site 1 and at 27 and 32 weeks of age site 2. The mean antibody titres of the vaccinated birds were above 3.5 at all the bleeding points after second vaccination at site 1 and above 3.9 at site 2. Peak antibody responses were obtained six weeks after V2 at site 1 and at the time of second vaccination at site 2. The mean antibody titres of the birds in control and vaccinated groups were significantly different at all bleeding time points after the first vaccination, at both sites, confirming the serological efficacy of the vaccination. The Applicant justified satisfactorily that although the German site (1) broilers breeders were treated for yolk sac infection at 35 week hatch point this treatment didn’t confound efficacy results.

In previous responses that a minimum protective IgY titre in eggs when measured by HIA assay can be correlated with reductions in lesions and mortality when chicks are challenged with *Clostridium perfringens* type A. If the egg titres measured by HIA are further examined, it can be seen that HIA titres in eggs are maintained above the minimum protective titre at all time points tested. From these trials onset of immunity was evident from 6 weeks post V2 and maintained for 26 weeks post V2 (the latest time point examined).

*Clostridium perfringens* was isolated from guts in control birds on both sites where NE was suspected. It was not isolated from chickens from vaccinated birds on either site.

At site 1 for both vaccinates and control birds, no clinical signs were observed for birds from hatch week 30. In birds from the 35 week hatch some signs were observed in <5% of both groups until day
9. From day 10 until the end of day 28 no signs were seen in either group. In birds from week 45 hatch some signs were observed in <5% of birds in the control group at the end of observation period (day 25-26).

At site 2 signs were in the control group and for 2 batches of chickens (week 27 and week 32) on days 24-25 and 25-26 when 80% of control birds had depression and ruffled feathers and inappetance was recorded in <5% of birds. “Wet litter” or moisture content ratio of faecal material did not correlate with clinical observations and the Applicant found interpretation difficult.

Conclusions: This study gave some overall information on the use of the vaccine in the field in terms of antibody development and passive transfer. There was a low level of field challenge which gave some information on the efficacy of the product in the face of challenge but this is of a supportive rather than a direct evidential nature.

Overall comments on field studies:
These can be considered of a supportive nature rather than direct evidence because of some confounding problems with serological measurements and no really substantial evidence of true challenge.

OVERALL CONCLUSION ON EFFICACY

A rationale for the product and a description of the disease and the involvement of *C. perfringens* in the disease syndrome was provided. The Applicant conducted a number of challenge studies to support the claims. Two field trials were reported both of which were conducted in Europe. The batches used in all efficacy studies contained between 2 and 3 TCP antigen input.

Overall the data submitted by the Applicant demonstrated that vaccination of chickens with Netvax induces the production of antibodies against *Clostridium perfringens* alpha toxin and that high titres of antibodies may be included in the yolk of eggs laid by vaccinated hens. As a result, the sera of the progeny of vaccinated hens contain significant titres of anti-alpha toxin antibodies for up to two to three weeks after hatching. The data also supported the claim for reduction of incidence and severity of lesions and of mortality due to necrotic enteritis.

In conclusion, the efficacy of Netvax was adequately demonstrated for the following:

“For the active immunization of chickens to provide passive immunisation against necrotic enteritis to their progeny, during the laying period. To reduce mortality and the incidence and severity of lesions caused by *Clostridium perfringens* Type A induced necrotic enteritis. Efficacy was demonstrated by challenge of chicks approximately three weeks after hatching.

The onset of passive transfer of immunity: 6 weeks following completion of the vaccination procedure
The duration of passive transfer of immunity: 51 weeks following completion of the vaccination procedure”
5. RISK-BENEFIT BALANCE

Benefit assessment

The claims for the vaccine have been adequately substantiated. The product has been shown to provide a direct benefit to the progeny of the vaccinated chickens in terms of protection against lesions induced by necrotic enteritis and a reduction in the incidence of mortality from the disease. It could also have an indirect benefit to the progeny of the vaccinated chickens in terms of a reduced exposure to antibiotics and therefore overall improvement in welfare of chicks in terms of health.

There may be an indirect benefit to the environment from the reduced risk of use of antibiotics reducing environmental exposure to these products.

Risk assessment

The schedule involves 2 vaccination steps and will result in injection site reactions of a moderate nature and moderate duration in the majority of vaccinated chickens. Accidental injection of an overdose would result in only a slight increase in the size and duration. Vaccination had no effect on weight gain. The limited nature of laboratory studies was supported by the results of field trials. Vaccination did not have a negative effect on reproductive performance when hens on the point of lay were vaccinated. The vaccine is not recommended for use during lay. There are no risks to the progeny.

There is a risk to the administrator of the product from self injection with an adjuvanted vaccine containing mineral oil. This has been adequately described on product literature.

The Applicant has also satisfactorily demonstrated that triethanolamine, which is included in the vaccine as an excipient but is not listed in the annexes to Council Regulation (EEC) No. 2377/90, is not pharmacologically active at the concentration included in the vaccine.

The antigenic component is inactivated and therefore does not pose a risk to the environment. The only potential hazard identified is from mercury present in the preservative, however, the pattern of use of the product is such that it is given in a highly controlled delivery system resulting in very low volume exposure to the environment. As the preservative will be processed within the body of the vaccinated animal before being excreted only extremely low levels will eventually reach the environment. The Applicant’s assessment of an overall negligible risk to the environment was accepted and no phase 2 assessment was considered necessary.

Benefit/risk evaluation

Overall as the claims of the product have been found sustainable, the CVMP can acknowledge the benefits of a reduction of mortality and reduced incidence and severity of lesions caused by necrotic enteritis covering the risk period of the young life of the chick. The above benefits outweigh the occurrence of moderate injection site reactions in the parent chicken which resolve after a reasonable length of time. The risks to the user of the product are significant if self injection occurs but warnings on the product literature indicate actions to be taken to prevent severe effects of self injection and therefore the risk is mitigated. There is no effective risk to the environment and there may be an indirect benefit from decreased use of antibiotics.

Conclusion

The overall benefit risk analysis is deemed positive with a sufficiently clear and complete SPC and product literature. Based on the original and complementary data presented, the Committee for Medicinal Products for Veterinary Use (CVMP) concluded that the quality, safety and efficacy of Netvax for Chickens were considered to be in accordance with the requirements of Council Directive 2001/82/EC, as amended.