EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

16 March 2017
EMA/142422/2016-Rev.1
Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

CVMP assessment report for Evalon (EMEA/V/C/004013/0000)

Common name: coccidiosis vaccine live for chickens

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5545
Send a question via our website www.ema.europa.eu/contact

Introduction

On 16 January 2015, the applicant LABORATORIOS HIPRA, S.A. submitted an application for a marketing authorisation to the European Medicines Agency (The Agency) for Evalon in accordance with Article 3(2)(b) of Regulation (EC) No 726/2004.

The eligibility to the centralised procedure was agreed upon by the CVMP on 11 September 2014. On the basis of summary information provided by the applicant the inclusion of the excipient Montanide IMS, at the time of agreement on eligibility to the centralised procedure, could be considered as a significant therapeutic innovation in providing protection. The rapporteur appointed was A.-M. Brady and the co-rapporteur appointed was B. Zemann.

Evalon is a live attenuated parasitic vaccine containing *Eimeria acervulina* strain 003 (332–450 oocysts/dose), *Eimeria brunetti* strain 034 (213–288 oocysts/dose), *Eimeria maxima* strain 013 (196–265 oocysts/dose), *Eimeria necatrix* strain 033 (340–460 oocysts/dose) and *Eimeria tenella* strain 004 (276–374 oocysts/dose). The pharmaceutical form is suspension and solvent for oral spray and is presented in cardboard boxes containing 1 vial of suspension (1,000 doses) and 1 vial of solvent (50 ml), 1 vial of suspension (5,000 doses) and 1 vial of solvent (250 ml), and 1 vial of suspension (10,000 doses) and 1 vial of solvent (500 ml). The route of administration is oral use with the intention that the chicks consume the vaccine orally by preening.

The applicant applied for the following indication: for active immunisation of chicks to reduce clinical signs, intestinal lesions and oocysts output of coccidiosis caused by *Eimeria acervulina*, *Eimeria brunetti, Eimeria maxima, Eimeria necatrix* and *Eimeria tenella*.

The dossier has been submitted in line with the requirements for submissions under Article 12(3) of Directive 2001/82/EC.

On 18 February 2016, the CVMP adopted an opinion and CVMP assessment report.

On 18 April 2016, the European Commission adopted a Commission Decision granting the marketing authorisation for Evalon.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided documents that set out a detailed description of the pharmacovigilance system (dated 2 March 2012) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Evalon is manufactured in the European Union (EU) by LABORATORIOS HIPRA, S.A. at three sites in Amer, Gerona, Spain. Secondary packaging and batch release for the EU will be carried out by LABORATORIOS HIPRA, S.A., Avda. La Selva 135, 17170 Amer, Gerona, Spain. A satisfactory certificate of Good Manufacturing Practice (GMP) compliance referring to inspection on 9 December 2013 has been provided. A satisfactory declaration of GMP compliance for the vaccine antigens, *Eimeria acervulina, E. brunetti, E. maxima, E. necatrix and E. tenella,* from the qualified person at the batch release site has also been provided. The qualified person's declaration is based on on-site audits.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system and the GMP certification of the manufacturing sites were considered to be in line with legal requirements.

Part 2 – Quality

Composition

Evalon is a live vaccine composed of sporulated oocysts derived from 5 precocious attenuated lines of *Eimeria* species which are suspended in a sterile phosphate buffered saline solution (PBS) and diluted in a solvent. Disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride and potassium chloride are included as excipients. The solvent constituents are Montanide IMS, vanillin, red AC and brilliant blue. The quantities per dose reflect the quantities tested by the in vitro procedures at the time of blending and this is acceptable. Information has been provided on the type of device to be used for delivery of the vaccine in particular details of the pressure of the device, volume per chick and droplet size.

Container

The product is filled into type I colourless neutral glass vials containing 7 ml (1,000 doses), 35 ml (5,000 doses) and 70 ml (10,000 doses). The containers are closed with type I polymeric elastomer rubber stoppers. These are then secured with an anodised aluminium cap. The solvent is filled into 50 ml, 250 ml and 500 ml polypropylene (PP) containers which are used to dilute the product to contain 1,000, 5,000 and 10,000 doses respectively. The solvent containers are closed with type I rubber stoppers and secured with an anodised aluminium cap. Details of the sterilisation method for the PP containers and rubber stoppers have been provided. Copies of sample certificates of analysis for the glass containers have been provided demonstrating compliance with European Pharmacopoeia (Ph. Eur.) monograph 3.2.1. For the plastic containers the applicant's and the suppliers' specifications should be aligned ensuring Ph. Eur. compliance. A copy of example certificates of analysis should be provided to confirm compliance. This is a post-authorisation condition to the marketing authorisation.

Development pharmaceutics

Evalon is a live vaccine composed of sporulated oocysts derived from 5 precocious attenuated lines of Eimeria species suspended in a sterile PBS. The product needs to be gently shaken and diluted with a solvent prior to coarse spray administration.

A description of the background to the disease, the life cycle of the *Eimeria* oocysts and the quality development of the vaccine, which is composed of five different *Eimeria* species (*E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix* and *E. tenella*), was provided. Avian coccidiosis is distributed worldwide with clinical signs ranging from decreased growth rate to a high percentage of sick birds with severe diarrhoea, an increased mortality percentage and decreased egg production.

The seven species of *Eimeria* internationally recognised to be the causative agents of avian coccidiosis in domestic fowl, can be differentiated by various characteristics. In considering their pathogenic properties and the fact that there is little or no cross-immunity between the different *Eimeria* species, five species should be considered for use in a vaccine intended for chickens with a long-life cycle: *E. acervulina, E. brunetti, E. maxima, E. necatrix and E. tenella.* These *Eimeria* species are commonly found in European farms and can cause severe disease in layers and breeders. Appropriate information from key poultry producing countries in Europe was provided to support the *Eimeria* species that are important for layers and breeders and thus confirm the relevance of the strains included in this vaccine.

Although there have been differing opinions on whether there are antigenic differences between strains of *E. maxima* there has been no conclusive evidence of antigenic subgroups with *E. maxima* and therefore only one *E. maxima* strain has been included in the vaccine Evalon (*E. maxima* strain 013) which is able to cross-protect against heterologous strains.

The vaccine is presented as a liquid form because the sporulated oocysts need to be in a liquid suspension to remain viable and immunogenic. Live sporulated oocysts (the infective form of the parasite) are required in a vaccine to initiate invasion and complete their life cycles. These are obtained from the faeces of infected chickens because it is not possible to use in vitro culture methods. Details of the sporulated oocyst concentration at the time of blending were included in the composition which is presented in the SPC rather than the quantity per dose as tested in the in vivo potency test and this was considered acceptable.

The excipients included in the vaccine were chosen to maintain a buffered isotonic solution and stability studies support their suitability.

The choice of solvent was based on past experience in the manufacture of a similar solvent for broilers. A description of vaccination under field conditions was provided. Reassurance that the encouragement of the chicks to preen/peck does not have any welfare concerns that are seen with pecking behaviour was provided.

The inclusion of an adjuvant is novel for this type of vaccine and its inclusion was appropriately justified. Montanide was selected because it was considered to enhance the efficacy of the active ingredients and provide a consistent degree of protection after a pathogenic *Eimeria* challenge.

No overages are added to cover losses of the active substances during the manufacture or during the shelf-life. A rationale for using the different packaging material was provided.

Method of manufacture

The method of manufacture is suitably described. It is essentially similar for each *Eimeria* strain with only minor differences to take account of their different characteristics. Four passages in coccidia-free SPF chicks are used to produce the vaccine antigens.

The manufacturing process established for the five *Eimeria* antigens is based on the "seed lot system", as indicated in the general monograph of the Ph. Eur. 0062 (Vaccines for veterinary use). It consists of a system of successive passages derived from one master seed lot. For each *Eimeria* species, the number

of passages from their master seed parasite (MSP) is identical and fixed. The MSP and all subsequent passages are only propagated in coccidia-free specified pathogen free chickens (SPF chicks).

The production process for each *Eimeria* species included in the vaccine is very similar. The chicks are inoculated with a suspension of sporulated oocysts. Their faeces are collected on a concrete period of time and the oocysts are recovered; then they are sporulated in the presence of a potassium dichromate solution and the resulting oocysts are re-inoculated to a new group of coccidia-free SPF chicks. This process is repeated a further three times and finally antigens are disinfected with sodium hypochlorite solution and processed. Production of the finished product involves mixing equal volumes of each strain with PBS and then aseptically filling into vials.

The solvent is produced by mixing brilliant blue (E-133), red AC (E-129) and vanillin with Montanide IMS. This is sterilised by filtration and aseptically filled into bottles.

Validation studies were provided for the manufacturing process for both the vaccine and the solvent and based on three consecutive production scale batches the consistent quality of the product in line with the proposed specifications was demonstrated. The solvent is manufactured at one batch size with the intention that larger batches may be produced following authorisation. A satisfactory validation study was presented supporting the sterilisation of the solvent using 0.2 µm filters.

A satisfactory validation study was also provided in support of the elimination of viral and mycoplasma contamination by the sodium hypochlorite treatment applied during the production process. Justification for the adequacy and completeness of the range of viruses tested in relation to the full range of possible avian extraneous agents has been provided.

The applicant has investigated the stability of the coccidia harvest of each *Eimeria* species from three batches and at two different temperatures: 5±3 °C and 12±3 °C. For *E. acervulina*, *E. maxima* and *E. tenella* the data provided demonstrate that the antigen bulks can be stored at either 5±3 °C or 12±3 °C for 2 months prior to blending and for 1 month for *E. brunetti and E. necatrix.* The vaccine is blended based on pre-storage concentration values.

Control of starting materials

Starting materials listed in the European Pharmacopoeia

The following starting materials which comply with the relevant the Ph. Eur. monograph are used: betamethasone sodium phosphate, dimethyl sulphoxide, disodium phosphate dodecahydrate, glycerol, potassium chloride, potassium dihydrogen phosphate, purified water, sodium bicarbonate, sodium chloride, SPF eggs and vanillin. Simethicone emulsion (USP 30/NF 25) is also used in the manufacture. Sample certificates of analysis for foetal bovine serum (FBS) have been provided although the applicant's and the suppliers' specifications should be aligned ensuring Ph. Eur. compliance. A copy of example certificates of analysis should be provided to confirm compliance. This is a post-authorisation condition to the marketing authorisation.

Starting materials not listed in the European Pharmacopoeia

Starting materials of biological origin

These include the five *Eimeria* strains, tryptose phosphate broth and coccidia-free SPF chicks.

All the strains were isolated in Spain from farms with evident clinical signs of coccidiosis. For all strains isolation, characterisation, purification and attenuation for precocity by serial passage in coccidia-free SPC chickens are adequately described.

The MSP and working seed parasite (WSP) were obtained after several passages in SPF coccidia-free chicks. Oocysts were recovered and sporulated. The resulting oocysts were used to prepare sporocysts that are stored in liquid nitrogen as the MSPs and WSPs.

Certificates of analysis of MSP and current WSP have been provided outlining the different tests, test conditions/samples, corresponding results and compliance with Ph. Eur. monographs. Controls carried out on the MSPs included sporocyst concentration (microscopic count), identity (PCR), viability, bacterial and fungal sterility (Ph. Eur.), freedom from mycoplasmas (Ph. Eur.), freedom from adventitious viruses (Ph. Eur.), freedom from avian leucosis virus (ALV), avian reticuloendotheliosis virus and chicken anaemia virus (CAV). WSPs are tested for sporozoite concentration, identity (PCR), viability and bacterial and fungal sterility. Certificates of analysis have been provided for each of the current WSPs, outlining the different tests, test conditions/samples, corresponding results and compliance with Ph. Eur. monographs.

Validation reports of PCR tests have also been provided confirming the sensitivity and specificity of these tests as well as the corresponding validations. The results are satisfactory.

Tryptose phosphate broth is an ingredient in the MEM Glasgow medium which is included in the freezing medium of the MSP and WSP for *E. acervulina*, *E. maxima* and *E. tenella*. The animal-origin raw materials are derived from porcine tissues and bovine milk. The quality control sheet, a product source information report (detailing the country of origin of the animal tissues) and an animal origin position statement are provided. An example certificate of analysis from the manufacturer has been provided.

Copies of sample certificates of analysis from each of the suppliers of the SPF eggs have been provided in order to confirm compliance with Ph. Eur. monograph 5.2.2.

Starting materials of non-biological origin

These are red AC (E-129), brilliant blue (E-133), Hank's balanced salt solution, MEM Glasgow medium, Montanide IMS, propionic acid, potassium dichromate and sodium hypochlorite. Quality control documents describing tests, specifications and results have been provided for all of the materials listed and are satisfactory. The selection of colouring agents and flavouring has been appropriately justified.

In-house preparation of media

Preparation of the following media is adequately described: freezing medium, propionic acid solution, saturated salt solution, potassium dichromate solution and sodium hypochlorite solution. The storage conditions proposed for each of them are acceptable.

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

The applicant has provided a TSE risk assessment for each of the biological starting materials in accordance with the Note for Guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products *(*EMA/410/01-Rev.3) and Commission Directive 1999/104/EEC.

Eimeria spp MSP/WSP: the *Eimeria* species used were isolated from infected chickens or their faeces. Since chickens are not considered to be susceptible to TSE infections the risk of TSE contamination from this source is negligible. FBS and tryptose phosphate broth are used in the freezing medium for the MSPs and WSPs. Only supplies of FBS covered by EDQM TSE certificates of suitability are used and the tryptose phosphate broth is unlikely to present any TSE risk (see below). The purity of the seed materials has been confirmed thus the risk of possible contamination of the final product could only come from the starting materials used during production or the production facilities itself. For vaccine production, the

Eimeria species are propagated in chickens which are not considered to be susceptible to TSE infections. No material of ruminant origin is included in the composition of the excipients or the solvent, nor used during the manufacturing process. With all stages of manufacture conducted according to GMP possible contamination from the facilities can be considered as negligible. The risk of transmitting TSE infection by use of this product is therefore negligible.

Tryptose phosphate broth is derived from bovine milk and porcine enzymes. Pigs are not considered to be a TSE-relevant species. The bovine derived material is obtained from healthy animals under the same conditions as milk for human consumption and is thus unlikely to present any TSE risk.

SPF chicks: birds are not considered to be a TSE-relevant species and therefore the risk of transmitting TSE infection by use of coccidia-free SPF chicks is considered to be negligible.

Overall, the documentation provided for all the materials of animal origin demonstrated their compliance with the Note for Guidance and the risk of transmitting TSE infection is considered to be negligible.

Control tests during production

Control tests carried out during production include oocyst counts (non-sporulated or sporulated at various stages during the process), sporulation rate, macroscopic observation of appearance, bacterial and fungal sterility, identity and purity by PCR and pH. Validation data for the sterility test have been provided. During production of the vaccine the integrity of filters is checked and volume is controlled on each batch.

During production of the solvent appearance, integrity of filters, bioburden, pH and volume are controlled.

Overall the in-process tests for the vaccine and solvent are described satisfactorily. Batch-to-batch consistency is demonstrated by the results of in-process controls for three batches, all of which met the required specifications.

Control tests on the finished product

Control tests carried out on each batch of the bulk vaccine include appearance, pH, concentration of sporulated oocysts, potency, concentration of excipients (residual sodium hypochlorite and potassium dichromate) and absence of mycoplasmas.

Control tests carried out on each batch of the filled vaccine include appearance, pH, concentration of sporulated oocysts, volume and bacterial and fungal sterility.

Acceptable data and justification have been provided to omit testing of extraneous agents on each batch of finished product.

Control tests on the bulk solvent include appearance, colour, pH, identification of colouring and flavouring agents and identification of the adjuvant (IR absorption spectrophotometry). On the filled solvent include appearance, bacterial and fungal sterility, pH and volume.

The finished product tests for the vaccine and the solvent are well described and the proposed limits are acceptable. Data of the analytical methods validation, when applicable, confirm their suitability.

The results of the analysis of three consecutive production runs indicate consistency between batches.

Stability

Stability results on three batches of finished product stored at 5±3 °C have been provided for up to 13 months. In addition data for batches stored at 12±3 °C for the first 3 months followed by storage at 5±3 °C for up to 10 months are provided. Storage of aged antigen is supported for 2 months for *E. acervulina*, *E. maxima and E. tenella* prior to blending and for 1 month for *E. brunetti and E. necatrix* with a subsequent shelf-life of 10 months.

In support of the stability of the solvent, data from three consecutive batches stored under two conditions of storage (5±3 °C or 20±5 °C) and on two batch sizes (50 ml and 500 ml) have been provided. Data have been presented for all control tests up to 24 months. On the basis of the data presented a shelf-life of 24 months at 5±3 °C is acceptable. A 5-day holding period prior to filling has been proposed and is acceptable.

An in-use stability study has been provided and supports the proposed 10 hours shelf-life after dilution.

Overall conclusions on quality

Evalon is a live vaccine composed of sporulated oocysts derived from 5 precocious attenuated lines of *Eimeria* species which are suspended in a sterile PBS solution and diluted in a solvent. Disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride and potassium chloride are included as excipients. The solvent constituents are Montanide IMS, vanillin, red AC and brilliant blue. The product is filled into type I colourless neutral glass vials containing 7 ml (1,000 doses), 35 ml (5,000 doses) and 70 ml (10,000 doses). The solvent is filled into 50 ml, 250 ml and 500 ml polypropylene (PP) containers to produce 1,000, 5,000 and 10,000 doses respectively when mixed with the respective vaccine doses. Both types of containers are closed with type I rubber stoppers and secured with an anodised aluminium cap. A post-authorisation condition to the marketing authorisation is established in relation to the plastic containers.

The manufacturing process for the vaccine is well described, with sufficient details. Validation studies were provided for the manufacturing process for both the vaccine and the solvent based on three commercial batches. Consistent quality of the product in line with the proposed specifications was demonstrated. Two other validation studies were presented supporting the sterilisation of the solvent.

Production and testing of the MSP and WSP are clearly described. Validation reports and certificate of analysis statements have been provided for all tests. The other starting materials of biological origin are acceptable. The risk of TSE contamination and the risk of extraneous agents contamination can be considered negligible.

A post-authorisation condition to the marketing authorisation is established in relation to the foetal bovine serum used in the preparation of MSP and WSP.

Generally the materials of non-biological origin are acceptable. Montanide IMS is included as an adjuvant in the solvent and specific details on this type of Montanide have been provided. In-house preparation of media is detailed satisfactorily.

Overall the in-process tests for the vaccine and solvent are described satisfactorily. A validation report has been provided for the PCR used to identify and purity check of the coccidia harvest and this is satisfactory.

The finished product tests for the vaccine and the solvent are well described and the proposed limits are acceptable. Data of the analytical methods validation, when applicable, confirm their suitability.

The results of the analysis of three consecutive production runs indicate satisfactory consistency between batches.

Stability data support a shelf-life for the vaccine of 10 months at 5±3 °C when antigens have been stored for 2 months (*E. acervulina*, *E. maxima* and *E. tenella*) or 1 month (*E. brunetti* and *E. necatrix*).

On the basis of the stability data presented for the solvent a shelf-life of 24 months when stored at 5±3 °C is acceptable. A 5-day holding period prior to filling has been proposed and is acceptable.

An in-use stability study has been provided and supports the proposed 10 hours shelf-life after dilution.

Part 3 – Safety

Safety documentation

Eleven laboratory trials and one multicentric field trial in support of the safety of the vaccine were provided. All the safety trials performed under laboratory conditions were carried out in accordance with Good Laboratory Practice (GLP) and all used 14-day-old chickens, which according to Ph. Eur. monograph 2326 is the age expected to be the most sensitive. However, the animals enrolled in the field trial were chicks of 1-day-old, which is the youngest recommended age for vaccination and claimed in the SPC.

Laboratory tests

Safety of the administration of one dose and of an overdose

The applicant carried out a single study to evaluate the safety of a single dose and an overdose of vaccine in SPF chickens. In this study, 14-day-old SPF chickens were vaccinated with a single dose and a ten-fold dose of vaccine. Administration was by oral gavage to ensure accurate administration of the complete dose. In order to ensure that the doses used were based on the maximum range per dose of sporulated oocysts that might be present in production batches the volumes used were 15% higher than the standard recommended dose. Individual body weight and feed consumption were recorded on study days 6 and 14. Three chickens were randomly taken from each study cage for necropsy and lesion scoring on study days 6, 7 and 14. Animals necropsied at day 7 were weighed again to obtain their final weights. Fresh faeces were collected on day 3 to day 9 from three cages of each treatment selected at random. A pool of litter faeces from each treatment was collected on days 7 and 14. Oocysts counts were performed from fresh and litter faeces. Other general parameters (clinical signs, mortality and faeces appearance) were monitored throughout the study.

No mortality or clinical signs were detected throughout the entire study period. Abnormal faecal appearance was observed in only one cage on a single day.

Statistical differences in lesion scores between groups were found in all sections of the intestine on day 6. Lesions were significantly different between groups A (PBS control) and C (10X overdose) in the duodenum. Lesions were significantly different between groups A and B (one dose) and between A and C in the upper and lower mid-intestine. Lesions were significantly different between groups A and C and B and C in caeca and rectums. On day 7, mean lesion scores for the different section of the intestine ranged from 0.03 to 0.28 for group B, and from 0.06 to 0.28 for group C. Mean lesion scores of all groups, on any day examined and for all sections of the intestine were not greater than 0.5. None of the lesions detected in the vaccinated animals, either vaccinated with one dose or one overdose, reached a score of

2. Differences between groups were only found in the caeca, where lesions were significantly different only between groups A and C.

Animals receiving treatment B (one dose) were not affected in terms of individual body weight. Animals of treatment C (10X overdose) had statistically lower individual body weight on day 6 post-inoculation than animals receiving treatments A (PBS) or B, but this difference was not statistically significant on day 14, indicating that the growth depression after an overdose of vaccine is transient and did not affect the overall performance of the birds over the extended production period. This is adequately described in section 4.10 of the SPC.

The elimination profile of oocysts and number of oocysts eliminated followed the expected curve for the vaccine and confirmed the administration of a dose and an overdose.

This GLP study therefore satisfactorily demonstrated the safety of the vaccine in two-week-old SPF chickens in compliance with Ph. Eur. monograph 2326. No long-lasting effects of the vaccine are anticipated.

Safety of the repeated administration of one dose

No studies on repeated administration of the vaccine were carried out because the vaccine is intended for only a single administration to birds before the onset of lay. This is considered acceptable.

Examination of reproductive performance

No studies on reproductive performance were carried out because the vaccine is intended for only a single administration to birds before the onset of lay. The SPC clearly indicates that the vaccine should not be administered to birds in lay or within two weeks before the onset of lay. This is considered acceptable.

Examination of immunological functions

No specific study has been carried out. Taking into consideration the nature and composition of this vaccine, there is no reason for suspecting an impairment of the immune system under the claimed conditions of use of the vaccine.

Special requirements for live vaccines

Spread of the vaccine strain

No specific studies on spread of the vaccine strains have been carried out. On the basis that spread and recycling of coccidia between litter and birds is an important feature of coccidial vaccines that is important for their efficacy this was considered acceptable. Since chickens are the only animals that are susceptible to the *Eimeria* species used in the vaccine there is no possibility of spread to other non-target bird species.

Dissemination in the vaccinated animal

No specific studies on dissemination of the strains in vaccinated birds have been carried out, on the basis that the trait of each *Eimeria* species relative to their affinity to a specific portion of the intestine is so well known that it is often used as a diagnostic feature of each individual species. The potential

contamination of organs other than the intestines has however been discussed, with reference to a dissemination study using another vaccine against *Eimeria* which shares three of the vaccinal strains *E. acervulina*, *E. maxima* and *E. tenella* with Evalon. In this study no parasites or lesions were observed in the spleen, liver, kidney, muscles, brain and bursa of Fabricius confirming the absence of dissemination of the vaccine strain in vaccinated chicks. Even though no data is available for *E. brunetti* or *E. necatrix* the CVMP accepted that there is no reason to expect that these *Eimeria* species would be any different to the three strains studied.

Reversion to virulence of attenuated vaccines

Two complementary studies for each vaccine strain were performed. In each case, the respective master seed was passaged five times through SPF chickens (studies for *E. necatrix*, *E. brunetti*, *E. acervulina*, *E. maxima* and *E. tenella*). Residual pathogenicity of the master seeds were then compared with the respective passaged isolates in studies for *E. necatrix*, *E. brunetti*, *E. acervulina*, *E. maxima* and *E. tenella*. In each study, two-week-old SPF chickens were inoculated by oral-gavage with the MSP or with its sporulated oocysts passaged 5 times (10,000 sporulated oocysts per bird). The chickens were observed for 21 days and the following parameters were monitored: clinical signs, mortality, faeces appearance, intestinal lesions, oocysts counts, weight evolution and feed consumption. Intestinal lesion scoring was carried out on days 6 or 7 following administration according to the optimum time for each species. Taken together these studies adequately satisfied the requirements of Ph. Eur. monograph 2326. No more than mild coccidial lesions were detected and there was no evidence of reversion to virulence for any of the strains during five passages in vivo.

Biological properties of the vaccine strain

The vaccine contains *Eimeria* strains attenuated for precocity. They were obtained from virulent parent strains by serial passage in chickens with the collection of oocysts from faeces within the first few hours after excretion. The vaccine strains have been demonstrated to be antigenic and not to cause adverse clinical signs in the target species, to have low reproductive potential and to spread from vaccinated to non-vaccinated animals without reversion to virulence.

Recombination or genomic reassortment of the strains

A detailed assessment of the risk of recombination between vaccine strains and field strains of *Eimeria* was provided. This concluded that although genetic recombination of Evalon strains (with a trait for precocious development) with field strains (drug-resistant normal *Eimeria* strains) is possible, any resulting oocysts would have the trait for precocious development. The resultant strains would be of equal or less pathogenic characteristics than the parent strains and that recombination, together with other factors, could also lead to a restoration of drug sensitivity within a given population of oocysts after vaccination.

Study of residues

The active ingredients being substances of biological origin intended to produce active immunity do not fall within the scope of Regulation (EC) No 470/2009 with regard to residues of veterinary medicinal products in foodstuffs of animal origin. The other substances included in the composition of this vaccine are either allowed substances for which table 1 of the annex to the Commission Regulation (EU) No 37/2010 indicates that no MRLs are required or are considered as not falling within the scope of Regulation (EC) No 470/2009. No specific residue studies are therefore required.

The withdrawal period is set at zero days.

Interactions

No specific compatibility studies have been done and therefore standard warnings are included in sections 4.8 and 6.2 of the SPC. Nevertheless, the potential interaction with other commonly administered vaccines used in the field within the same time frame as Evalon has been discussed. A range of other vaccine targeting diseases such as Mareks, Newcastle, IBD and *Salmonella* were used in the field studies following vaccination with Evalon and no adverse events reported due to interactions were observed. Section 6.2 of the SPC also includes a warning that anticoccidial drugs should not be used for at least three weeks following vaccination because this could interfere with vaccine replication which is important for vaccine efficacy.

Field studies

A single Good Clinical Practice (GCP) compliant multicentre field trial was carried out to assess both the safety and efficacy of the vaccine under field conditions. A total of 171,254 chicks of one day of age (74,154 layers and 97,100 breeders) were used, distributed into several farms in Spain and Germany at the beginning of the study. A total of four rearing farms and five laying farms were used. The trials were randomised, double blind and controlled. A positive parallel control group vaccinated with an authorised vaccine against *Eimeria* was used. A total of 85,628 birds were vaccinated with Evalon and 85,626 were vaccinated with the already authorised vaccine.

The vaccines were administered to chicks at one day of age by coarse spray. The feed was free of anticoccidial agents. The only treatment with anticoccidial effect was amprolium used after a coccidiosis outbreak in the control group of one farm. Any adverse events that appeared after the administration of the vaccine, in any of the animals, at any time of the study were recorded. Other parameters recorded were mortality and weight changes. Any alteration in the appearance of faeces was evaluated from the day of vaccination until seven weeks of age. Necropsies were performed on 15 animals from each group on days 6, 7 and 24 to examine for intestinal lesions.

Neither the Evalon vaccinated nor the control animals showed any adverse effect attributable to the administration of the product. There were no post-vaccination general or local reactions, alteration of faeces appearance or deaths attributable to the vaccination with Evalon. There were no significant differences in weight gain between the groups. Only mild intestinal lesions were detected in necropsied birds and when significant differences were seen, the lesion scores were higher in the control group than in the Evalon group. None of the groups vaccinated with Evalon vaccine showed changes on productive parameters such as egg production and hatchability.

The field trial therefore provided adequate confirmation of the safety of the vaccine when administered to one-day-old chicks by coarse spray in the field.

User safety

A user safety risk assessment for the vaccine was provided in accordance with the CVMP guideline for user safety for immunological veterinary medicinal products (EMEA/CVMP/IWP/54533/2006).

The parasites of the genus *Eimeria* are not zoonotic agents and they are not pathogenic for humans and therefore do not pose any risk for the person handling the product or the person who is in contact with vaccinated animals. All excipients included in the composition are common excipients used in veterinary vaccines and do not pose a risk for the final user.

In case of improper use or breakage of the containers the main routes of exposure would be dermal and ocular. If contact with the skin or eyes occurred, toxic effects caused by the components of the product are unlikely to occur.

In case of accidental ingestion during vaccination, adverse effects are unlikely to occur because the active ingredients are not zoonotic agents and the excipients present in the product are non-toxic.

In conclusion, no hazard has been identified. Even in the worst case scenario no effect is expected if an exposure to the product occurs.

Environmental risk assessment

An environmental risk assessment in accordance with the Note for guidance on environmental risk assessment for immunological veterinary medicinal products (EMEA/CVMP/074/95) was provided.

A phase I environmental risk assessment has been provided, the main points of which were:

Parasites of the genus *Eimeria* are characterised by their rigid and strong host specificity. The risk of the vaccine strains included in Evalon to be transmitted to non-target species is therefore negligible.

Vaccinated chicks can shed oocysts of each *Eimeria* species included in the vaccine. The capacity of the *Eimeria* strains included in the vaccine to survive in the environment is not expected to be any different from other *Eimeria* strains. This does not involve any risk to non-vaccinated target animals, since the vaccine strains included in Evalon have been attenuated and the absence of reversion to virulence has been demonstrated.

Genetic recombination of Evalon strains with field strains is possible but the resultant strains would be of equal or less pathogenic characteristics than the parent strains and moreover, recombination together with other factors could also lead to a restoration of drug sensitivity within a given population of oocysts after vaccination.

Apart from the antigen, the rest of the vaccine components are very well-known excipients widely used in pharmaceutical formulations. They are generally regarded as non-toxic at the low concentrations used and do not constitute a risk to the environment.

It is therefore concluded that the risk to the environment from the use of this vaccine is negligible and that no specific control measures are needed in addition to the general management recommendations of poultry farms and the precautions included in the product information concerning the handling and disposal of unused veterinary medicinal product or waste materials derived from the use of such product.

Based on the data provided the ERA can stop at Phase I. Evalon is not expected to pose a risk for the environment when used according to the SPC.

Overall conclusions on the safety documentation

The route of administration used in the laboratory safety studies, oral gavage, differs from the recommended route of administration by spray. However, the intention of the coarse spray administration is that the chicks consume the vaccine orally by preening and therefore the use of oral gavage to ensure that each chick received the full dose of vaccine is adequately justified.

A single laboratory safety study on 14-day-old SPF chickens to investigate the safety of a single dose and an overdose of vaccine administered via the oral route was provided. Safety of an overdose was demonstrated in line with Ph. Eur. requirements but laboratory demonstration of safety of a single dose was not strictly in accordance with the requirements. The deviation from the strict requirements of Ph.

Eur. chapter 5.2.6, taking into account that the applicant has addressed the specific Ph. Eur. requirements of monograph 2326 and also the provisions of Directive 2010/63/EC which requires that the number of animals used in experiments is reduced to a minimum without compromising the objectives, has been appropriately justified. The strategy to investigate the inherent safety of a single dose or overdose of the vaccine for the target species is acceptable and it would not be justifiable to require an additional single dose study in one-day-old SPF birds.

It is therefore accepted that this study satisfactorily demonstrated the safety of the vaccine in two-week-old SPF chickens in compliance with Ph. Eur. monograph 2326. No long-lasting effects of the vaccine are anticipated. The possibility of a transient reduction in live weight gain is indicated in section 4.10 of the SPC.

The lack of studies on repeated administration of the vaccine, on reproductive performance, on immunological functions and on dissemination or spread of the vaccine strains was adequately justified.

To address residual pathogenicity and reversion to virulence two Ph. Eur. compliant complementary studies for each vaccine strain were provided. No more than mild coccidial lesions were detected and there was no evidence of reversion to virulence during five passages in vivo.

A single GCP compliant multicentre field trial was carried out to assess both the safety and efficacy of the vaccine under field conditions.

Neither the Evalon vaccinated nor the control animals showed any adverse effect attributable to the administration of the product. There were no post-vaccination general or local reactions, alteration of faeces appearance or deaths attributable to the vaccination with Evalon. There were no significant differences in weight gain between the groups. Only mild intestinal lesions were detected in necropsied birds and when significant differences were seen, the lesion scores were higher in the control group than in the Evalon group. None of the groups vaccinated with Evalon vaccine showed changes on productive parameters such as egg production and hatchability.

The field trial therefore provided adequate confirmation of the safety of the vaccine when administered to one-day-old chicks by coarse spray in the field.

An assessment of the risk of recombination between vaccine strains and field strains of *Eimeria* concluded that although genetic recombination of Evalon strains with field strains is possible, the resultant strains would be of equal or less pathogenic characteristics than the parent strains and that recombination, together with other factors, could also lead to a restoration of drug sensitivity within a given population of oocysts after vaccination.

No specific residue studies are required. The withdrawal period is set at zero days.

No specific risks to the end user or to the environment were identified. Based on the data provided the ERA can stop at phase I. A suitable user warning is proposed for the SPC.

Part 4 – Efficacy

Introduction and general requirements

Avian coccidiosis is distributed worldwide. Signs of coccidiosis range from decreased growth rate to a high percentage of sick birds with severe diarrhoea, an increased mortality percentage and consequently, decreased egg production. Decreased feed intake and increased water consumption may also accompany outbreaks.

Generally, seven species of *Eimeria* that affect the *Gallus gallus* are internationally accepted to be the causative agents of avian coccidiosis. These species are: *E. acervulina, E. brunetti, E. maxima*, *E. mitis, E. necatrix, E. praecox* and *E. tenella.* However, taking into account their pathogenic properties and that there is little or no cross immunity between the different *Eimeria* species, five species should be considered as essential when dealing with a vaccine intended for long life cycle chickens: *E. acervulina, E. brunetti*, *E. maxima, E. necatrix* and *E. tenella*. All these species are commonly found in European farms.

Evalon is a live attenuated vaccine which contains 5 different *Eimeria* species: *E. acervulina, E. maxima, E. tenella, E. brunetti* and *E. necatrix.* It is presented as a suspension in a sterile PBS solution and diluted with a solvent (containing Montanide IMS, Brilliant Blue, Red AC and vanillin). The vaccine is recommended for administration once by coarse spray to 1-day-old chicks. It is intended for the active immunisation of future layers and breeders to reduce intestinal colonisation, intestinal lesions and clinical signs of coccidiosis caused by those *Eimeria.*

Three laboratory efficacy studies have been conducted to evaluate different efficacy parameters and establish the onset of immunity (OOI) and duration of immunity (DOI). In addition one combined safety and efficacy field study was carried out with sites in Spain and Germany.

The target intestinal area for each *Eimeria* species were assessed in the efficacy studies: *E. acervulina* invades the epithelial cells of the duodenal loop but infection may spread to the upper mid-intestine*, E. maxima* and *E. necatrix* typically parasitise the upper and lower mid-intestine including the duodenum or caeca, respectively, *E. brunetti* is generally located in the lower mid-intestine and rectum but also can be found in proximal areas of the caeca and *E. tenella* is mainly found in the caeca.

Laboratory trials

Two batches of Evalon were used in the efficacy trials. All of the animals included in the efficacy trials were vaccinated with 5.95 µl of Evalon, instead of the standard vaccine dose (7 µl). This was to provide a minimum range per dose of sporulated oocysts 15% lower than the concentration of sporulated oocysts contained in a standard dose, thereby achieving a dose of vaccine containing the minimum titre of sporulated oocysts for each species that might be included in a commercial dose.

The *Eimeria* species used to perform the challenge were different (heterologous) from the ones included in the vaccine. The vaccinal strains are all derived from Spanish field isolates whereas the challenge strains were isolated in the United Kingdom at the coccidia reference laboratory of Houghton Poultry Research Station in Compton (UK). The infectious process is rapid (4–7 days) and the day of necropsy and lesion scoring for *E. acervulina, E. brunetti* and *E. necatrix* was 6 days after challenge and for *E. maxima* and *E. tenella* 7 days after challenge.

Onset of protection

Onset of protection was investigated in two trials. In study SPF chicks vaccinated at one day of age were challenged with *E. acervulina, E. maxima* and *E.* tenella 21 days after vaccination. The design of study was similar but vaccinated chicks were challenged with *E. brunetti* and *E. necatrix* 21 days after vaccination. A control group of SPF chicks treated with sterile PBS via coarse spray was also included in both studies. These two studies together were designed to investigate the OOI for each of the *Eimeria* species in the vaccine.

In the first study a total of 126 SPF chicks were treated and challenged of which 63 were vaccinated with the test product (group A) at one day of age and another 63 were treated with sterile PBS (group B). In

each of the treatment groups 3 weeks after vaccination 27 birds were challenged with *E. brunetti*, another 27 with *E. necatrix* and 9 were challenged with PBS.

In the second study a total of 180 SPF chicks were treated and challenged of which 90 were vaccinated with the test product (group A) at one day of age and another 90 were treated with sterile PBS (group B). In each of the treatment groups 3 weeks after vaccination 27 birds were challenged with *E. acervulina*, another 27 with *E. maxima,* 27 with *E. tenella* and 9 were challenged with PBS.

In both studies the following parameters were monitored after challenge: intestinal lesions, individual weight and growth rate, feed consumption, clinical signs, mortality, faeces appearance and oocysts counts.

All remaining birds after necropsy were euthanised on day 14 after challenge.

Intestinal lesions indicative of the coccidial challenge species were evaluated based on the scoring system described in the Ph. Eur. monograph 2326. According to this monograph, for challenges with *E. tenella*, *E. acervulina*, *E. necatrix*, *E. maxima* or *E. brunetti*, at least 80% of the vaccinated birds should have no or minimal lesions in the intestine and no bird should have a lesion score of 4. Following challenge for each of the coccidial species in the vaccine, lesion scores (in the target area) were significantly lower in the vaccinates compared to the controls with >80% of vaccinated birds with a lesion score of ≤1. Two birds had a score of 4, i.e. died during the study, but there was no evidence that the deaths were associated with the challenge infection and thus the results indicated that the vaccine was in compliance with the Ph. Eur. monograph. Animals were weighed during the challenge phase and although there were not always significant differences between groups, growth rate was significantly different on at least one time point for each of the coccidial species included in the vaccine. Clinical signs, including faecal appearance and mortality were described and at least 80% of the vaccinated animals were observed with mild signs of disease which were less marked than those in the control group thus in compliance with the Ph. Eur. monograph. No statistical comparisons were made. The number of oocysts excreted per bird during the challenge phase was described. For all coccidial species included in the vaccine, the average number of oocysts excreted was reduced in the vaccinated group compared to the control group by greater than 60%. An OOI of 3 weeks is supported by these studies.

Maternally derived antibodies

No studies on the influence of maternally derived antibodies (MDA) on vaccine efficacy have been presented. Given that vaccination is targeting 1-day-old chicks and that laying hens can transfer maternal antibodies to their hatchlings via the egg yolk, an understanding as to the influence of MDA is necessary. Laboratory studies included the use of SPF birds (status doesn't include freedom from antibodies against *Eimeria)* which were not contaminated with *Eimeria* parasites. However, no serology data is presented to understand whether there were any antibodies against *Eimeria* thus no impact of maternal antibodies could be assessed. Field studies included chicks from previously vaccinated breeders although given the lack of field challenge it is not possible to understand the effect of any maternal antibody interference. Nevertheless, it was argued that chicks used in the field studies should have had maternal antibodies against *Eimeria* species following confirmation that all parent breeder flocks involved were confirmed to be vaccinated with other authorised vaccines against *Eimeria*. The fresh faeces oocyst counts demonstrated the ability of vaccinal oocysts to replicate and complete their life cycle in the host and also the litter oocyst counts were undetectable by 5–6 weeks after vaccination. Oocyst excretion kinetics confirmed acquired immunity and the development of full immunity which would not have happened if MDA has a negative impact on vaccine uptake, replication and recycling of oocysts (necessary for development of immunity with this type of vaccine). Similar oocyst count profiles were obtained in the laboratory efficacy studies after vaccination and following challenge oocyst excretion was significantly reduced in vaccinated groups compared to the non-vaccinated controls. Given the ubiquitous nature of

Eimeria species and the usage of other authorised *Eimeria* vaccines, it is concluded that MDA are unable to stop replication of the vaccinal strain and immunity. Summaries of studies with other vaccines against *Eimeria* were presented in which the influence of MDA was studied and it was concluded that the elimination of oocysts post vaccination and intestinal lesions were similar regardless of whether MDA were present or not. Furthermore a detailed bibliographic review relating to MDA has been provided to support the lack of maternal interference. No information has been provided on the serological status of the chicks used in the laboratory study, nevertheless given the extensive justification and further information from studies with an authorised vaccine against *Eimeria*, it can be accepted that the presence of MDA does not impact the development of immunity against *Eimeria* species.

Duration of immunity

A DOI for all of the *Eimeria* species in the vaccine was investigated in a single laboratory study.

895 SPF chicks were vaccinated with the test product (group A) at one day of age and other 895 SPF chicks were treated with sterile PBS (group B). For each of the challenge periods the birds were further randomly allocated to 6 different groups and they were challenged with the various *Eimeria* species or PBS 14 weeks after vaccination (17 weeks for *E. acervulina*), 28 weeks after vaccination, 40 weeks and 60 weeks after vaccination. Each challenge (at 14 weeks and at 40 weeks) was performed in a total of 144 animals from each treatment group (A and B). At week 28 the challenge was performed in a total of 96 animals from each treatment group (A and B) and at week 60 in a total of 110 animals from each treatment group (A and B).

The efficacy parameters studied were the same as those assessed in the OOI study: intestinal lesions indicative of the coccidial challenge species were evaluated and statistically compared; body weights and growth rates were evaluated and statistically compared; clinical signs were described in line with the methods used for the OOI study and oocyst excretion was described. The efficacy parameters were assessed over a 14-day period post challenge except for the challenge at 28 weeks after vaccination where the observation period was 7 days.

Following challenge 14 weeks post-vaccination (17 weeks for *E. acervulina*) for each of the coccidial species in the vaccine the following observations were made:

- For all *Eimeria* species, intestinal lesion scores (in the target areas) were significantly lower in the vaccinates compared to the controls with >80% of vaccinates birds with a lesion score of ≤1 and no birds had a score of 4 thereby meeting the requirements of Ph. Eur. monograph 2326.
- Weights were variable post-challenge, however growth rate was significantly different between vaccinated and controls on at least one time point for each of the coccidial species included in the vaccine.
- Clinical signs, including faecal appearance and mortality were evaluated but not statistically compared. No abnormal clinical signs were observed in any of the vaccinated animals post challenge whereas clinical signs were seen in the unvaccinated controls challenged with all of the *Eimeria* species.
- The number of oocysts excreted per bird during the challenge phase was evaluated following challenge the average oocysts excreted was reduced in the vaccinated group compared to the control group by 75.7%–100% depending on species.

For the challenge after 28 weeks only the evaluation of intestinal lesions was reported. As not all efficacy parameters were assessed at this time point, a DOI of 28 weeks cannot be considered.

Following challenge 40 weeks post-vaccination for each of the coccidial species in the vaccine the following observations were made:

- For all *Eimeria* species, intestinal lesion scores (in the target areas) were significantly lower in the vaccinates compared to the controls with >77% of vaccinates birds with a lesion score of ≤1 and no birds had a score of 4. This is just below the >80% requirements of Ph. Eur. monograph 2326.
- Weights were variable post challenge, however growth rate was significantly different between vaccinated and controls on at least one time point for each of the coccidial species included in the vaccine.
- Clinical signs, including faecal appearance and mortality were evaluated but not statistically compared. Abnormal clinical signs were observed in both vaccinated animals and unvaccinated controls challenged with *E. maxima*, *E. necatrix* and *E. tenella* although to a lower degree in the vaccinated animals. No clinical signs were observed in the vaccinated group challenged with *E. acervulina* and *E. brunetti,* however clinical signs were observed in the control group
- The number of oocysts excreted per bird during the challenge phase was evaluated following challenge the average oocysts excreted was reduced in the vaccinated group compared to the control group by 86.9%–100% depending on species.

Following challenge 60 weeks post-vaccination for each of the coccidial species in the vaccine the following observations were made:

- For all *Eimeria* species, intestinal lesion scores (in the target areas) were significantly lower in the vaccinates compared to the controls with >73% of vaccinates birds with a lesion score of ≤1 and no birds had a score of 4. This is lower than >80% requirements of Ph. Eur. monograph 2326.
- Weights and growth rates were variable post challenge.
- Clinical signs, including faecal appearance and mortality were evaluated but not statistically compared. Abnormal clinical signs were observed in both the vaccinated animals and the unvaccinated controls challenged with all of the *Eimeria* species. Clinical signs in the vaccinated groups were not reduced to a lower extent than the controls for *E. acervulina* and *E. tenella.*
- The number of oocysts excreted per bird during the challenge phase was evaluated following challenge the average oocysts excreted was reduced in the vaccinated group compared to the control group by 65.1%–100% depending on species.

In the absence of statistical comparison of the clinical signs between vaccinated and unvaccinated control animals but clear difference in the faecal appearance between both groups after challenge, a DOI of 60 weeks with reduction of clinical signs (diarrhoea), intestinal lesions and oocyst excretion following challenge with all five *Eimeria* species is acceptable.

Field trials

A single combined safety and efficacy multicentre field study was carried out. No unvaccinated control group was included in this study and comparison was made to an already authorised vaccine. The conclusions that can be drawn from this study are therefore limited. On all sites, elimination of oocysts following vaccination followed a typical pattern for the attenuated strains and was reduced by three weeks, indicating similar levels of vaccine 'take' for both vaccines. Although all of the sites are stated to have had a history of coccidiosis, field infection was confirmed on only one site, when the group vaccinated with the already authorised vaccine became infected with *E. necatrix*. However, considering the ubiquitous occurrence of coccidia, it is likely that the birds would have been exposed to field infection

on all sites, and the fact that only one outbreak of disease occurred during the study could be reflective of the efficacy of both vaccines. There was little difference between the two vaccines except on one site when the difference was in favour of Evalon. This study therefore provides some support for the efficacy of Evalon. No further information could be gained on field exposure from the field study however Evalon has been used in the field under special permit in the UK, Germany, France, Denmark, Portugal and Spain with no lack of efficacy reported.

Overall conclusion on efficacy

Three laboratory efficacy studies which were conducted to an acceptable standard and well designed to establish the OOI and DOI were provided. In addition one multicentre field study (4 farms) was conducted to support the laboratory studies.

Two studies to evaluate the OOI and one study to evaluate the DOI were conducted, using appropriate challenge strains for each coccidial species included in the vaccine. In all laboratory efficacy studies the vaccine was administered by coarse spray to 1-day-old chicks which is the recommended age for this vaccine. The passage level of the antigen for production is a fixed value from the MSP; there is not a range. All the animals included in the laboratory efficacy trials were vaccinated with 5.95 µl of the test product, instead of the standard vaccine dose (7 µl) to achieve a minimum range per dose of sporulated oocysts of 15% lower than the concentration of sporulated oocysts contained in a standard dose.

Individual body weights, feed consumption, clinical signs, mortality and oocyst counts were assessed following vaccination and confirmed the homogeneous groups and the appropriate administration of the treatments as indicated by no contamination of the control group and that the vaccination group had a peak of oocyst elimination which followed a curve within normality. The efficacy parameters were assessed over a 14-day period post challenge.

An OOI of 3 weeks is supported by these studies.

In the study to evaluate the DOI birds were challenged 14, 28, 40 and 60 weeks after vaccination.

The data provided supports a DOI of 60 weeks with reduction of clinical signs (diarrhoea), intestinal lesions and oocyst excretion following challenge with all five *Eimeria* species.

No studies on the influence of MDA on vaccine efficacy have been presented. Nonetheless, considering the justification and further information from studies using an already authorised *Eimeria* vaccine, it can be accepted that the presence of MDA does not impact the development of immunity against *Eimeria* species.

A single combined safety and efficacy multicentre field study was carried out. No unvaccinated control group was included in this study and comparison was made to an already authorised vaccine against *Eimeria*. The conclusions that can be drawn from this study are therefore limited. On all sites, elimination of oocysts following vaccination followed a typical pattern for the attenuated strains and was reduced by three weeks, indicating similar levels of vaccine 'take' for both vaccines. Considering the ubiquitous occurrence of coccidia, it is likely that the birds would have been exposed to field infection on all sites, and the fact that only one outbreak of disease occurred during the study is probably reflective of the efficacy of both vaccines. There was little difference between the two vaccines except on one site when the difference was in favour of Evalon. This study therefore provides some support for the efficacy of Evalon. No further information could be gained on field exposure from the field study however Evalon has been used in the field under special permit in the UK, Germany, France, Denmark, Portugal and Spain with no lack of efficacy reported.

The product has been shown to be efficacious for active immunisation of chicks from 1 day of age to reduce clinical signs (diarrhoea), intestinal lesions and oocysts output associated with coccidiosis caused by *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix* and *E. tenella*.

The data provided in relation to reduction of clinical signs other than diarrhoea was considered insufficient by the CVMP to support this claim.

Part 5 – Benefit-risk assessment

Introduction

Evalon is a live attenuated vaccine containing precocious strains of *E. acervulina*, *E. brunetti, E. maxima, E. necatrix* and *E. tenella*. It is intended for active immunisation of chicks to reduce clinical signs, intestinal lesions and oocysts output of coccidiosis caused by infection with these species. The active components are similar to other authorised vaccines against coccidiosis in chickens but the inclusion of an adjuvant (Montanide) is novel for this type of product.

The application has been submitted in accordance with Article 12(3) of Directive 2001/82/EC.

Benefit assessment

Direct therapeutic benefit

Evalon is a fixed multivalent vaccine containing attenuated strains of the five most important coccidia for long-lived chickens (layers and breeders).

Data have been presented that show a benefit of inclusion of Montanide IMS as an adjuvant in enhancing the efficacy of the active ingredients.

Well-designed laboratory trials conducted to acceptable standards demonstrated that the product is efficacious in the following indication:

For active immunisation of chicks from 1 day of age to reduce clinical signs (diarrhoea), intestinal lesions and oocysts output associated with coccidiosis caused by *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix* and *E. tenella*.

Onset of immunity: 3 weeks post-vaccination.

Duration of immunity: 60 weeks post-vaccination in an environment that permits oocysts recycling.

Lack of statistical comparison of the clinical signs observed during the laboratory trials between vaccinated and unvaccinated control animals prevents the CVMP from accepting that Evalon is indicated to reduce the clinical signs associated with coccidiosis caused by the five *Eimeria* species as initially proposed by the applicant. The only clinical sign for which the data demonstrate there is a reduction is diarrhoea.

The data provided in relation to reduction of clinical signs other than diarrhoea was considered insufficient by the CVMP to support this claim.

Additional benefits

Coloured and flavoured excipients included in the vaccine encourage oral uptake of the vaccine by preening of sprayed birds.

Evalon contains live attenuated strains of five species of coccidia. These are precocious strains that complete their development cycle in the vaccinated chickens and are shed into the litter before colonisation reaches a level that would cause disease. Recycling of sporulated oocysts from the litter further enhances efficacy.

There is currently one authorised vaccine that contains all of the coccidial species included in Evalon that is widely available throughout Europe. Evalon therefore would increase the range of available treatment possibilities in the EU.

Coccidial infections are widely treated with anticoccidial drugs which can have a potential risk of inducing resistance. Use of this vaccine to enhance resistance to infection might therefore have an indirect benefit in reducing the use of anticoccidial drugs.

Risk assessment

Main potential risks are identified as follows:

Quality:

Evalon is manufactured under GMP conditions and with a good range of in-process tests that should ensure consistency. Information on development, manufacture and control of the antigens and finished product (vaccine and solvent) has been presented in a satisfactory manner. Batch-to-batch consistency and stability has been demonstrated.

For the target animal:

As Evalon contains live oocysts that colonise the intestine of vaccinated birds there is a risk that they could cause disease. This risk could be enhanced if the strains reverted to virulence during the recycling that is important for vaccine efficacy. However, safety studies demonstrated only a low risk of adverse effects. There was no evidence of reversion to virulence during five passages in vivo.

All of the laboratory safety studies were carried out using 14-day-old chickens, which is considered to be the most sensitive age for adverse effects from coccidiosis. This is somewhat older than the minimum age recommended for the vaccine (one-day-old). However, large numbers of one-day-old chicks were vaccinated in a large scale field trial and no adverse effects were noted. It is therefore concluded that the attenuated strains of the vaccine are safe for the vaccinated chickens when used as recommended.

If development of the vaccine strains is not allowed to progress naturally or if recycling of shed oocysts is prevented then efficacy may be compromised. Therefore, anticoccidial drugs should not be used in vaccinated chickens.

Administration of Evalon in accordance with SPC recommendations is generally well tolerated by target animals.

For the user:

The CVMP concluded that user safety for this product is acceptable when used according to the SPC recommendations. Standard safety advice is included in the SPC.

For the environment:

Vaccine strains of coccidia are shed into the environment, and this is enhanced by repeated recycling through chickens in the house. There is therefore a significant risk that other species, including humans, could be exposed to the vaccine strains. However, the species of coccidia included in the vaccine are considered to be highly host specific for chickens and therefore induction of disease in other species or in humans in contact with the vaccinated birds is unlikely.

Since coccidia are ubiquitous and commonly present in poultry houses, there is a high likelihood that recombination between vaccine strains and virulent strains could occur. However, in this event the resultant strains would be of equal or less pathogenic characteristics than the parent strains. All of the vaccine strains are sensitive to common anticoccidial drugs and therefore could not introduce resistance to virulent strains. It is also possible that recombination, together with other factors, could lead to a restoration of drug sensitivity within a given population of oocysts after vaccination.

Evalon is not expected to pose a risk for the environment when used according to the SPC.

For the consumer:

A residue study is not required. The withdrawal period is set at zero days.

Risk management or mitigation measures

Taking into account the good safety profile of this vaccine no specific mitigation measures are proposed. Only general user safety measures are proposed for the SPC.

To ensure that efficacy is not compromised by inadequate development of the vaccine strains the SPC contains a warning that chickens must be strictly floor-reared in the first three weeks after vaccination. Furthermore a warning not to use anticoccidial drugs for at least 3 weeks following vaccination of chickens is also included.

Appropriate information has been included in the SPC and other product information to inform on the potential risks of this product relevant to the target animal, user, environment and consumer and to provide advice on how to prevent or reduce these risks.

The withdrawal period is set at zero days.

Evaluation of the benefit-risk balance

The product has been shown to be efficacious for active immunisation of chicks from 1 day of age to reduce clinical signs (diarrhoea), intestinal lesions and oocysts output associated with coccidiosis caused by *E. acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix* and *E. tenella*.

An OOI of three weeks has been demonstrated and DOI 60 weeks post-vaccination in an environment that permits oocysts recycling.

The data provided in relation to reduction of clinical signs other than diarrhoea was considered insufficient by the CVMP to support this claim.

The potential impact of MDA on vaccine efficacy has been discussed and any potential interference discounted.

The formulation and manufacture of Evalon is well described and batch-to-batch consistency and stability have been demonstrated. Stability after mixing with the solvent has been investigated and confirmed.

The product is well tolerated by the target animals and presents a low risk for users, consumers and the environment when used as recommended and appropriate warnings have been included in the SPC.
A sufficient withdrawal period has been set.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the CVMP concluded that the application for Evalon is approvable since these data satisfy the requirements for an authorisation set out in the legislation (Regulation (EC) No 726/2004 in conjunction with Directive 2001/82/EC).

The CVMP considers that the benefit-risk balance is positive and, therefore, recommends the granting of the marketing authorisation for the above mentioned medicinal product.