15 June 2022
EMA/602317/2022
Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for Evanovo (EMEA/V/C/005819/0000)

Vaccine common name: Coccidiosis vaccine live for chickens

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

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Introduction

The applicant Laboratorios Hipra, S.A. submitted on 21 April 2021 an application for a marketing authorisation to the European Medicines Agency (the Agency) for Evanovo, through the centralised procedure under Article 3(2)b of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 10 December 2020 as the applicant showed that the product constitutes significant technical innovation.

At the time of submission, the applicant applied for the following indications: for the active immunisation of chickens to reduce clinical signs, intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria acervulina, Eimeria maxima, Eimeria praecox* and *Eimeria tenella*.

Evanovo is a live coccidiosis vaccine, including attenuated strains of *Eimeria acervulina*, *Eimeria maxima*, *Eimeria praecox* and *Eimeria tenella* as active substances. The target species is chicken. The product is intended for administration by the in ovo route.

Each Evanovo dose (0.006 ml) of undiluted vaccine contains:

Eimeria acervulina, strain 044	598 - 809*
Eimeria maxima, strain 013	352 - 476*
Eimeria praecox, strain 007	235 - 317*
Eimeria tenella, strain 004	221 - 299*

* Number of sporulated oocysts derived from precocious attenuated lines of coccidia, according to *in vitro* procedures of the manufacturer at the time of blending.

Evanovo is presented in packs of one vial containing 6 ml (1000 doses), 12 ml (2000 doses), 24 ml (4000 doses), 30 ml (5000 doses), 48 ml (8000 doses) and 60 ml (10000 doses).

The solvent HIPRAHATCH is recommended for use with Evanovo.

The rapporteur appointed is Mary O'Grady and the co-rapporteur is Manuela Leitner.

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

On 15 June 2022, the CVMP adopted an opinion and CVMP assessment report.

On 27 July 2022, the European Commission adopted a Commission Decision granting the marketing authorisation for Evanovo.

Marketing authorisation under exceptional circumstances

Not applicable.

Scientific advice

Not applicable.

MUMS/limited market status

Not applicable.

Part 1 - Administrative particulars

Detailed description of the pharmacovigilance system

The applicant has provided a detailed description of the pharmacovigilance system (Version 00, date 14/01/2019) 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 Union or in a third country.

Manufacturing authorisations and inspection status

Evanovo is manufactured in the European Union (EU) by LABORATORIOS HIPRA, S.A. at three sites in Amer, Gerona, Spain.

Secondary packaging and batch release take place at LABORATORIOS HIPRA, S.A. Avda. La Selva, 135 Amer, Spain.

Manufacturing authorisation issued on 26th January 2021 by the Spanish competent authority covers the manufacturing activities at these sites. Good Manufacturing Practice (GMP) certification, which confirms the date of the last inspection of the sites (21st September 2018) and shows that the sites are authorised for the manufacture and batch release of such veterinary dosage forms, has been provided.

A GMP declaration for the active substances manufacturing sites was provided from the Qualified Person (QP) at the EU batch release site. The declaration has taken into consideration the GMP certificates available for the active substance sites issued by the Spanish competent authority following inspection of the sites on 21st September 2018.

A copy of the GMP certificate for each active substance manufacturing site issued by the Spanish authorities following inspection on 21st September 2018 is also provided.

Overall conclusions on administrative particulars

The detailed description of the pharmacovigilance system was considered in line with legal requirements.

The GMP status of the active substance(s) and of the finished product manufacturing sites has been satisfactorily established and are in line with legal requirements.

Part 2 – Quality

Chemical, pharmaceutical and biological/microbiological information (quality)

Qualitative and quantitative particulars of the constituents

Qualitative and quantitative particulars

Evanovo is a live vaccine, which contains as active substances sporulated oocysts derived from four precocious attenuated lines of the following *Eimeria* species: *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella*.

The vaccine suspension contains the sporulated oocysts suspended in a sterile phosphate buffered saline solution containing disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, potassium chloride and purified water. Polysorbate 80 is included as an excipient in the vaccine suspension. Each vaccine dose is formulated to contain a standard amount of sporulated oocysts of each *Eimeria* strain; the ranges referred to in SPC section 2 reflect the variability of the oocyst counting procedure.

The solvent HIPRAHATCH is recommended for use with Evanovo. This solvent contains disodium phosphate dodecahydrate, potassium dihydrogen phosphate, sodium chloride, potassium chloride and water for injection.

The vaccine is intended to be available in multidose presentations but contains no preservative. The claimed 10 hours in-use shelf life is satisfactorily justified in the stability section of the dossier.

The vaccine is recommended for associated use with another avian vaccine Gumbohatch. The claimed 2 hours associated in-use shelf life is satisfactorily justified in the stability section of the dossier.

Container and closure

The vaccine suspension is filled into colourless type I glass vials of the following sizes:

10 ml for the 1000 doses (containing 6 ml)

20 ml for the 2000 doses (containing 12 ml)

50 ml for the 4000 and 5000 doses (containing 24 ml and 30 ml respectively)

100 ml for the 8000 and 10000 doses (containing 48 ml and 60 ml respectively)

The vials comply with European Pharmacopoeia (Ph. Eur.) chapter 3.2.1 requirements.

The HIPRAHATCH solvent is filled into colourless polypropylene plastic (PP) bags. The solvent presentations are 250 ml bags containing 200 ml of solvent, 500 ml bags containing 400 or 500 ml of solvent, and 1000 ml bags containing 800 ml or 1000 ml of solvent. The PP material meets the requirements of Ph. Eur. 3.1.3 and Ph. Eur. 3.1.6 chapters.

Type I rubber stoppers composed of a polymeric elastomer are used as closures for the vaccine containers and comply with Ph. Eur. chapter 3.2.9.

Details of the sterilisation method for the containers (vaccine and solvent) and rubber stoppers have been provided. All sterilising conditions are in accordance with the reference conditions specified in Ph. Eur. chapter 5.1.1 and are acceptable.

Caps of anodised aluminium are used to ensure the correct closure of the stoppers for the vaccine containers.

The method of closure and opening of the container/closure is adequately described.

The pack/container sizes are consistent with the vaccination schedule and intended use.

Product development

Evanovo is proposed for administration *in ovo*. The applicant stated that at the time of application there were no other live attenuated vaccines against avian coccidiosis for *in ovo* administration in the

European market. An explanation and justification for the composition and presentation of the vaccine has been provided.

It is explained that avian coccidiosis is distributed worldwide with clinical signs of coccidiosis ranging from decreased growth rate to a high percentage of sick birds with severe diarrhoea, an increased mortality percentage and decreased egg production.

Seven species of *Eimeria* are considered the causative agents of avian coccidiosis in domestic fowl: *E. acervulina, E. brunetti, E. maxima, E. mitis*, *E. necatrix, E. praecox* and *E. tenella.* Each species can be differentiated by characteristics such as oocyst size, reproductive capacity, pathogenicity, immunogenic properties, pre-patency period, minimum sporulation time and several molecular characteristics.

The four species included in Evanovo i.e. *E. acervulina, E. maxima, E. praecox* and *E. tenella* are considered important for a vaccine intended for short-lived birds such as commercial broilers. *E. praecox* infections generally appear in the first weeks of life resulting in a negative effect on weight gain – as such, *E. praecox* infections have a higher impact in short lived birds (e.g. commercial broilers) than in long-lived birds (e.g. layers or breeders).

For the other three *Eimeria* species, detection rates of >70% for farms surveyed in France in 1994 are quoted in a literature publication provided. While more recent reporting rates for the European Union are not provided as these three strains are included in HIPRA's currently authorised HIPRACOX BROILERS (authorised for >10 years), EVALON (authorised April 2016) and EVANT (authorised February 2019) vaccines, it is accepted that *E. acervulina, E. maxima* and *E. tenella* strains are representative of current EU *Eimeria* strains relevant for broiler chicks and as such their inclusion in Evanovo is justified. *E. acervulina* 044 strain included in Evanovo is a new *E. acervulina* strain compared to the one included in the other HIPRA's currently authorized coccidia vaccines. Evanovo does not include the *Eimeria* species *E. mitis,* as according to the applicant there is a low prevalence of the strain in the European Union.

The *Eimeria* vaccine strains have been selected so that they have reduced or no pathogenicity. Attenuation of pathogenicity is achieved by repeated passage in chickens with selection for early appearance of oocysts. By selection for precocious development, populations can be selected with an important reduction of pre-patent periods (i.e. the time from ingestion of sporulated oocysts to emergence of oocysts in the faeces) and a significant reduction of pathogenicity.

The attenuation of the *Eimeria* species in Evanovo is satisfactorily described and involved inoculation of the purified parental strains to coccidia-free SPF chicks to obtain the initial passage (PO). The first oocysts eliminated were re-inoculated to new coccidia-free SPF chicks to perform the next passage. A number of similar successive passages were performed until populations of oocysts with greatly reduced pre-patent times and pathogenicity compared to the parental strains were obtained.

The sporulated oocysts need to be in a liquid suspension to remain viable and immunogenic: therefore, a suspension was chosen as the pharmaceutical form. The diluent used for the vaccine is a sterile phosphate buffered solution (PBS) with the addition of polysorbate 80, which is the same diluent used with the company's EVANT vaccine.

The HIPRAHATCH solvent is a sterile PBS solution with no immunostimulant effect. The components of the solvent are the same as those used in the vaccine suspension with the exception of polysorbate 80, which is not included.

All excipients are well known pharmaceutical ingredients and their quality complies with Ph. Eur. standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SPC.

Vaccine batches are blended to contain a fixed amount of sporulated oocysts of each *Eimeria* species hence no overages are used in vaccine blending. Considering the fixed amount of sporulated oocysts of each species, the formulation of batches used during clinical studies is the same as that intended for marketing.

An acceptable rationale for the vaccine and solvent packaging materials used was provided.

The proposal not to test routine vaccine batches for the extraneous agents listed in Ph. Eur. 5.2.5 'Management of extraneous agents in immunological veterinary medicinal products' is acceptable, as Ph. Eur. 2326 Coccidiosis vaccine live for chickens monograph allows for the omission of this testing where the oocysts are disinfected by a validated procedure. The sodium hypochlorite disinfection procedure used in the production of each *Eimeria* species in Evanovo was validated to reduce the virus titre of representative contaminating viruses by at least 6 $\log_{10}$ in accordance with Ph. Eur. 5.2.5. Furthermore, the seed materials have been satisfactorily tested for freedom from relevant extraneous agents listed in Ph. Eur. 5.2.5. Additional information was requested and further provided by the applicant during the responses to questions.

Description of the manufacturing method

The manufacturing method and the in-process controls for the production of sporulated oocysts of each of the *Eimeria* components of the vaccine are similar with only minor differences to take account of their different characteristics. Several passages in coccidia-free SPF chicks are used to produce the vaccine active substances as described below.

The manufacturing process established for the four 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 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 after a concrete period of time and the oocysts are recovered; then they are sporulated 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 volumes of each strain with the excipients and then aseptically filling into vials.

Production data are provided from batches of each *Eimeria* species, which support the consistency of manufacture.

Data supporting the storage time for intermediates are provided.

Batch protocols are provided for batches. These demonstrate that the manufacturing process is capable of producing batches of acceptable quality in a reproducible and consistent manner.

Data from batches are provided to support the consistency of the manufacturing process. Acceptable validation data for the sterilising filtration step are provided.

The HIPRAHATCH solvent is produced by mixing all the components. Once these are mixed, the solution is sterilised before aseptic filling. Data from three batches are provided to support the consistency of the manufacturing process. The HIPRAHATCH solvent is aseptically filled into PP bags before being placed into a plastic overpouch and terminally sterilised by autoclaving.

Production and control of starting materials

Starting materials listed in pharmacopoeias

The following starting materials listed in a pharmacopoeia are used and the correspondent chapters and monographs are indicated in brackets: betamethasone sodium phosphate (Ph. Eur. 810), dimethyl sulphoxide (Ph. Eur. 763), disodium phosphate dodecahydrate (Ph. Eur. 118), foetal bovine serum (Ph. Eur. 2262), glycerol (Ph. Eur. 496), polysorbate 80 (Ph. Eur. 428), potassium chloride (Ph. Eur. 185), potassium dihydrogen phosphate (Ph. Eur. 920), purified water (Ph. Eur. 008), simethicone emulsion (USP 42/NF 37), sodium bicarbonate (Ph. Eur. 195), sodium chloride (Ph. Eur. 193), SPF eggs (Ph. Eur. 5.2.2) and water for injection (Ph. Eur. 169).

Representative certificates of analysis are provided supporting compliance with the pharmacopoeial standards. The foetal bovine serum is gamma irradiated with a minimum dose of 30 kGy.

The nature of the raw materials, controls and treatments applied guarantee sterility of the vaccine and absence of introduction of any extraneous agent.

Specific materials not listed in a pharmacopoeia

Starting materials of biological origin

These include the four *Eimeria* strains (*E. acervulina* 044, *E. maxima* 013, *E. praecox* 007, *E.tenella* 004), tryptose phosphate broth and coccidia-free SPF chicks.

All strains were isolated from Spanish poultry farms.

For all four strains the isolation, characterisation, purification and attenuation for precocity by serial passage in coccidia-free SPF chickens are adequately described.

Master Seed Parasites (MSP) were prepared for each of the strains as follows: the oocysts from the last passage of the attenuation process were identified. Sporocysts were prepared from these oocysts inoculated into coccidia-free SPF chicks, the faeces collected and sporulated. The resulting sporulated oocysts were used to prepare sporocysts that were stored frozen in liquid nitrogen as the MSP. Protocols are provided outlining the steps involved in the preparation of each MSP – these are sufficiently detailed.

The tests done on each of the MSPs included sporocyst concentration, identity by polymerase chain reaction (PCR), viability, bacterial and fungal sterility, freedom from mycoplasmas and extraneous agents testing. Satisfactory validation of the PCR identity test was provided and sufficient justification was provided to ensure that the individual strains of *E. acervulina* are satisfactorily differentiated during manufacturing.

Each MSP was tested for the absence of extraneous agents listed in Ph. Eur. 5.2.5 annex I for avian species (poultry and additional list for chickens).

A certificate of analysis is provided for each MSP outlining the satisfactory results. The tests for adventitious viruses using embryonated hen's eggs, chicken kidney cells and chicks were done according to Ph. Eur. 2.6.24 and a crystal violet stain is used to detect cytopathic effect (CPE) as an alternative to the stains specified in the monograph. The NAT methods used have been satisfactorily validated and acceptable details of the positive controls used in the PCR identity tests are provided.

Working Seed Parasites (WSP) were prepared by inoculation of the MSP into coccidia-free SPF chicks followed by recovery of oocysts, which were then sporulated.

Each WSP is tested for sporocyst concentration, identity (PCR), viability and bacterial and fungal sterility. Certificates of analysis are provided for each WSP, which are satisfactory.

Tryptose phosphate broth (TPB) is a component of the MEM Glasgow medium included in the freezing medium of the MSP for several *Eimeria* species. The animal-origin raw materials are derived from porcine tissues and bovine milk. A certificate of analysis, an animal origin position statement and a satisfactory risk assessment in accordance with Ph. Eur. chapter 5.2.5 are provided.

Coccidia-free SPF chicks used for MSP, WSP and successive passages are hatched from SPF eggs. Representative certificates of analysis from each of the suppliers confirm compliance with Ph. Eur. chapter 5.2.2 on Chicken flocks free from specified pathogens for the production and quality control of vaccines. An acceptable justification is given for the tests done on the chicks between hatching and their use for Eimeria production to confirm the absence of specific pathogens.

The risk of contamination of the vaccine with extraneous agents due to the above referenced biological origin starting materials is considered to be negligible on the basis that all MSPs have been tested for freedom from the extraneous agents listed in Ph. Eur. 5.2.5 annex I for avian species (poultry and additional list for chickens), a satisfactory risk assessment for Tryptose Phosphate Broth in accordance with Ph. Eur. 5.2.5 is provided and as the SPF chicks are from eggs meeting Ph. Eur. 5.2.2 requirements. On this basis, the applicant's proposal that routine vaccine batches will not be tested for extraneous agents can be accepted, particularly as Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens allows for omission of extraneous agent testing where there is a validated disinfection procedure, and vaccine batches tested to date were negative for extraneous agents.

A transmissible spongiform encephalopathies (TSE) risk assessment for each of the biological starting materials in accordance with the Note for Guidance (NfG) on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products *(*NfG EMA/410/01 rev.3) and Commission Directive 1999/104/EEC is provided, which supports the negligible risk of TSE transmission as follows:

Eimeria species MSPs/WSPs:

Each *Eimeria* species was isolated from infected chickens or their faeces – chickens are not considered susceptible to TSE infection. The foetal bovine serum used in the freezing medium of the MSPs and WSPs is covered by European Directorate for the Quality of Medicines (EDQM). Valid TSE certificates of suitability have been provided. Tryptose phosphate broth, which is included in the freezing medium for several MSPs, is derived from bovine milk and porcine tissues, which are considered to represent a negligible risk. Chickens, which are not considered susceptible to TSE infection, are used for vaccine production. No material of ruminant origin is included in the composition of the excipient or the solvent or used in the manufacturing process.

Tryptose phosphate broth (TPB):

The animal origin materials in TPB are porcine enzymes and bovine milk. Pigs are considered non-TSE relevant species (NfG EMA/410/01) and the bovine milk 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 a TSE-relevant species.

Starting materials of non-biological origin

These are Hank's balanced salt solution (HBSS), MEM Glasgow medium, potassium dichromate, propionic acid and sodium hypochlorite. Acceptable quality control documents describing tests, specifications and results have been provided.

In-house preparation of media and solutions consisting of several components

Preparation of the following media is described: freezing medium, propionic acid solution, saturated salt solution, potassium dichromate solution, sodium hypochlorite solution, PST VC dilution medium and PBS. Details of the qualitative and quantitative composition and the treatment and storage of the media are satisfactorily described.

Control tests during the manufacturing process

The applicant presented in-process data for the manufacture of three consecutive antigen bulks. During the manufacture of the antigen, the following tests are carried out for each of the four *Eimeria* species contained in each of the bulks: total oocysts before floatation, total sporulated oocysts and percentage of sporulation after sporulation, concentration of sporulated oocysts and percentage of sporulation before blending, identity/purity (PCR), appearance, sterility and pH. Additional testing carried out during manufacturing of the vaccine is filter integrity testing before and after each sterilising filtration of PBS and polysorbate solution, residual sodium hypochlorite and volume control on each batch of vaccine during aseptic filling. Test descriptions and the limits of acceptance were presented. Total oocysts and sporulated oocysts and percentage of sporulation are counted and validation of the counting method is given in Part 2E. Sterility testing is in line with Ph. Eur. 2.6.1. Overall, based on the data obtained, the in-process tests are deemed sufficient to control all the critical steps in the antigen manufacturing process. The test methods for the in-process controls are satisfactorily validated.

The control tests performed on three batches of diluent HIPRAHATCH solvent are appearance, pH, sterility and volume control. The bacterial and fungal sterility for each batch of HIPRAHATCH solvent is satisfactorily performed according to the Ph. Eur. 2.6.1. The in-process tests are deemed sufficient to control the critical steps in the HIPRAHATCH solvent manufacturing process.

Control tests on the finished product

The applicant presented detailed description and validation of the control testing on three finished product batches. The European Pharmacopoeia monograph Coccidiosis vaccine (live) for chickens (2326) requires that batch testing includes identification, sterility testing, mycoplasma, extraneous agents testing, sporulated oocysts count and potency testing, these requirements are met. Finished product testing on the bulk for Evanovo includes: appearance, pH, concentration of sporulated oocysts, concentration of sodium hypochlorite, detection of mycoplasma and batch potency. Control testing on the finished product includes appearance, pH, volume, sterility and concentration of sporulated oocysts. Concentration of sporulated oocysts is performed by microscopical examination using a validated counting method

Sterility is determined according to the Ph. Eur. 2.6.1, which is acceptable. Mycoplasma testing was performed in line with the Ph. Eur. 2.6.7 on the bulk before filling, which is acceptable. The applicant proposed to omit the tests to demonstrate freedom from extraneous agents in line with the Ph. Eur.

specific monograph Coccidiosis vaccine (live) for chickens (2326). The freedom of the starting material from extraneous agents has been sufficiently demonstrated. Also, the sodium hypochlorite oocyst disinfection procedure has been validated to reduce representative contaminating viruses by at least 6 log_{10} and all batches tested to date meet Ph. Eur. 5.2.5 requirements. Overall, the negligible risk of the presence of extraneous agents in the vaccine has been demonstrated and the omission of extraneous agent testing of the finished product is considered acceptable in line with Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens.

The batch potency test is described, validated, and includes appropriate acceptance criteria in line with the requirements for immunogenicity in Ph. Eur. monograph 2326 Coccidiosis vaccine (live) for chickens. The data provided show that all of the required specifications were fulfilled for all *Eimeria* species and repetitions performed during the validation of the potency test. The test has been shown to acceptably discriminate between potent and subpotent batches. Results from routine batches are shown to be statistically significant when treatment groups are compared, vaccinated versus non-vaccinated challenged, as opposed to results from subpotent batches where treatment groups (vaccinated versus non-vaccinated challenged) give results that are not statistically significant.

The potency test is also used to confirm the presence of oocysts; verifies the counting process i.e. the amount of total sporulated oocysts contained in a known volume of the vaccine per dose; confirms the identity by identifying the presence of each species in the blend and viability.

Batch-to-batch consistency

The applicant presented final product data for the manufacture of three consecutive final product batches. During the manufacture of the antigen the following tests are carried out (total oocysts, total sporulated oocysts, percentage of sporulation, concentration of sporulated oocysts percentage before blending, sterility, identity and pH) for harvest for each *Eimeria* species. Test descriptions and the limits of acceptance were presented. Final batch testing of the 3 bulks met all of the required specifications for appearance, pH, concentration of sporulated oocysts, concentration of sodium hypochlorite, mycoplasma and batch potency. Sterility is tested in line with Ph. Eur. 2.6.1 and mycoplasma testing is in line with Ph. Eur. 2.6.7. The concentration of sporulated oocysts for each of the 3 bulks was within the required limits. The batch potency was determined for the 3 final bulks and for each of the 4 *Eimeria* species and met all of the required specifications. The final batches (x 3 batches of 1000, 2000, 5000 and 10,000 doses) from each of the 3 bulks also met the required specifications for appearance, pH, volume, sterility and concentration of sporulated oocysts.

In-process control testing (appearance, pH, sterility and volume control) of the diluent HIPRAHATCH SOLVENT bulks is acceptable, all results are within the required limits.

Overall, the batch-to-batch consistency data provided is acceptable.

Stability

For the active ingredient:

Data on the stability of the bulk active ingredient were provided for three coccidia harvests of each *Eimeria* species. The results of the tests from the stability study of the antigens support the proposed active substance shelf life of 3 months. Data on one finished product batch produced with antigens stored for 3 months before blending had been provided demonstrating stability for this batch up to 18 months. The stability data provided supports the proposed antigen shelf life of 3 months.

For the finished product:

The applicant has provided stability data for three consecutive batches up to at least 15 months. All results met with required specifications. Therefore, a finished product shelf life of 12 months is considered acceptable in line with Ph. Eur. 0062.

Real-time stability of the HIPRAHATCH solvent to be used with Evanovo is the same as the solvent used for GUMBOHATCH and real-time data has already been assessed with GUMBOHATCH. Acceptable stability data for HIPRAHATCH solvent supports the proposed shelf life up to 36 months.

The applicant is proposing an in-use shelf life of 10 hours for Evanovo, based on data already approved for their other similar vaccines. The proposed 10-hour in-use shelf life is considered acceptable in this instance.

The applicant provided the results of in-use stability study for Evanovo mixed with the GUMBOHATCH vaccine for proposed associated use. An in-use period of 2 hours was demonstrated with two batches of each vaccine, in line with the Guideline on data requirements to support in-use stability claims for veterinary vaccines (EMA/CVMP/IWP/250147/2008), and results were acceptable.

Overall conclusions on quality

Evanovo is a live vaccine that consists of a suspension and solvent for suspension for injection. Evanovo contains as active substances sporulated oocysts derived from the following *Eimeria* species: *Eimeria acervulina, Eimeria maxima, Eimeria praecox* and *Eimeria tenella*.

The qualitative and quantitative particulars of the vaccine suspension and solvent and the containers are described adequately. The necessary certificates are provided.

The applicant gives a detailed description of the development of the vaccine strains and its manufacturing process. Summaries of studies, which were performed in the course of the development of the vaccine, and studies on attenuation of the active substances are provided.

The method of production of the sporulated oocysts used as active substances is provided. Overall, an adequate description of each process is given and the procedure for disinfection of the oocysts is validated for removal of relevant extraneous agents. Data supporting the storage of the sporulated oocysts prior to vaccine blending is provided.

Vaccine manufacture involves mixing of standard volumes of the sporulated oocysts of each strain and the excipients under aseptic conditions followed by aseptic filling into vials. Data from three batches support the manufacture of consistent batches of acceptable quality.

The production of the HIPRAHATCH solvent is described in sufficient detail. The solution is sterilised before aseptic filling. The HIPRAHATCH solvent is aseptically filled into PP bags before being placed into a plastic overpouch and terminally sterilised by autoclaving. Acceptable validation data for the terminal sterilisation is provided. Satisfactory data supporting the consistency of the manufacturing process are given.

The representative certificates of analysis provided for all pharmacopoeial grade materials support the quality of each material. Foetal bovine serum is irradiated at a minimum of 30kGy before use, in line with Ph. Eur. 2262.

A detailed description of the preparation and testing of each master and working seed parasite is given, which is acceptable. Overall, the testing of the seed materials is satisfactory and validation is provided for the testing methods used.

For the other biological origin starting materials (tryptose phosphate broth (TPB) and coccidia-free SPF chicks) a satisfactory risk assessment according to Ph. Eur. 5.2.5 has been carried out for TPB and the SPF chicks are hatched from SPF eggs meeting Ph. Eur. 5.2.2 requirements.

Data have been presented to give reassurance on TSE safety in accordance with NfG EMA/410/01 rev.3.

In-process data for the manufacture of three consecutive antigen bulks for each *Eimeria* spp. used in the vaccine production and three batches of HIPRAHATCH solvent were presented. Test descriptions and the limits of acceptance were presented. Total oocysts and sporulated oocysts, and percentage of sporulation are counted using a validated counting method. Sterility testing is in line with Ph. Eur. 2.6.1. Based on the data provided, the in-process tests are deemed acceptable to control all of the critical steps in the manufacturing process. In general, the test methods for in-process controls are satisfactorily validated. Based on the review of the data on quality, the control of Evanovo is considered acceptable.

The applicant presented finished product testing data for three consecutive batches. The testing performed meets the batch testing required in the Ph. Eur. monograph 2326 Coccidiosis vaccine (live) for chickens. Identification to confirm the presence of oocysts in the batch of vaccine is determined using a validated counting method, the potency test is used to confirm the presence of oocysts for the *Eimeria* species and their identity. Sterility and mycoplasma are appropriately determined according to Ph. Eur. 2.6.1 and Ph. Eur. 2.6.7 respectively. The extraneous agents testing is proposed to be omitted from the FP testing in line with the Ph. Eur. monograph 2326 Coccidiosis vaccine (live) for chickens. Acceptable validation data has been submitted and the omission of these testing is acceptable.

The batch potency test is described satisfactorily. Validation of the potency test showed that all of the required specifications were met for all *Eimeria* species. The test has been shown to acceptably discriminate between potent and subpotent batches. In-process testing for the manufacture of three consecutive final product batches and the limits of acceptance were provided. In-process control testing of the three diluent bulks were provided and met all of the required limits. Sterility and mycoplasma were tested in line with Ph. Eur. 2.6.1 and Ph. Eur. 2.6.7, respectively. In-process testing and final antigen batch testing for each of the 4 *Eimeria* species met all of the required specifications.

The applicant also provided acceptable data for 3 manufacturing scale finished product batches including a batch produced using the aged antigens stored for 3 months. Overall, the batch-to-batch consistency data provided is acceptable. The real time stability data for three batches of each coccidia harvest and one batch of the finished product produced with the aged antigen support the proposed 3 months storage of the active substance for manufacturing of the finished product with the proposed shelf life of 12 months.

Real time stability study for 3 consecutive batches filled into the smallest (10 ml) and largest (100ml) presentations was submitted to support a proposed product shelf life of 12 months. Sufficient data were presented to support the proposed shelf life.

A shelf life of 36 months is supported for HIPRAHATCH solvent.

A 10-hour in use shelf life is considered acceptable. The applicant proposed in-use shelf life for Evanovo mixed with the GUMBOHATCH vaccine of 2 hours.

Part 3 – Safety

Introduction and general requirements

Evanovo is a live vaccine containing 4 different *Eimeria* species, intended to stimulate active immunity against avian coccidiosis caused by *Eimeria acervulina*, *E. maxima*, *E. praecox* and *E. tenella.* The strains included have been attenuated for precocity, by repeated passage in chickens with selection for early appearance of oocysts. The time from ingestion of sporulated oocysts to emergence of oocysts in the faeces is termed the pre-patent period and differs between each *Eimeria* species. By selection for precocious development, populations can be selected with a reduction of pre-patent periods and a significant reduction of pathogenicity.

A single dose of the vaccine is intended for in ovo use in 18-day-old embryonated chicken eggs. The vaccine suspension is diluted prior to administration in the solvent provided for use with the vaccine, HIPRAHATCH (sterile PBS), in order to administer a final dose volume of either 0.1 ml or 0.05 ml for use in automated egg injection machines calibrated to administer either of the two dose volumes. One dose of vaccine is 6 µl in each case, only the volume of solvent differs. Instructions for the appropriate volumes of solvent to use for vaccine dose presentations of 1,000, 2,000, 4,000, 5,000, 8,000 and 10,000 doses are included in the SPC, depending on final required dose volume of 0.05 ml or 0.1 ml per dose.

The active substances of Evanovo are:

Strain	**Range of sporulated oocysts for standard dose***
Eimeria acervulina, strain 044	598 – 809*
Eimeria maxima, strain 013	352 – 476*
Eimeria praecox, strain 007	235 – 317*
Eimeria tenella, strain 004	221 – 299*

* The range established correlates to ± 15% of the standard concentration per dose (fixed concentration)

A full safety file in accordance with Article 12(3) has been provided. The safety of the immunological veterinary medicinal product has been investigated in accordance with the requirements of Directive 2001/82/EC, as amended. In addition, Ph. Eur. monograph 5.2.6 'Evaluation of safety of veterinary vaccines and immunosera', and the specific requirements outlined in Ph. Eur. monograph 2326 Coccidiosis vaccine (live) for chickens have been taken into account in order to demonstrate the safety of the vaccine.

Safety documentation

Nine laboratory trials and one multicentric field trial were carried out to assess the safety of Evanovo. The nine pivotal laboratory studies and the field studies were conducted according to GLP standards and GCP guidelines, respectively. In addition, two laboratory trials which investigated the safety of associated use were carried out.

Specific requirements for the investigation of the safety of the vaccine outlined in Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens specify the use of chickens of the category that is expected to be the most sensitive, i.e. 14-day-old chickens. However, since the vaccine is intended to be used only in ovo to 18-day-old embryonated eggs, the safety of vaccination has been evaluated in this category of target species. This approach is considered acceptable.

The investigation of the safety of the vaccine was performed at the maximum content of sporulated oocysts that will be present in a dose. Depending on the purpose of the study, the master seed lot (master seed parasite; MSP) or the vaccine was inoculated. In all safety laboratory trials to study both residual pathogenicity and increase in virulence, in order to assure that each chick receives the dose established in each protocol, the MSP was administered by oral gavage (directly into the oesophagus of each bird). This is in line with the requirements of Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens. However, since in ovo is the only recommended method of vaccination, in the laboratory safety to investigate the safety of one dose and an overdose and in the field trial, this was the only method used.

One batch of vaccine has been used in the laboratory safety studies. This batch was stated to have been manufactured in accordance with the manufacturing process described in the dossier and at the same manufacturing facilities that will be used for future production batches, containing coccidian harvest from each *Eimeria* species at the least attenuated passage level that will be present in a batch of vaccine.

During the safety studies, clinical signs, mortality, weight, faeces appearance, oocyst production and intestinal lesion scores were monitored.

The scoring of intestinal lesions followed the system described by Johnson and Reid (1970), incorporated in Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens, for the species-specific lesions visible in the intestine for *E. acervulina*, *E. maxima* and *E. tenella*. For *E. praecox*, which is known not to induce macroscopic lesions (as per Ph. Eur. 2326), the intestinal tract is required to be examined for microscopic evidence of infection such as demonstration of oocysts or developing oocysts; for these two strains the presence of parasites, macroscopic lesions and histological changes were evaluated.

The scoring of clinical signs of coccidiosis was performed as follows:

0: Animals with normal appearance. Respond to stimuli, eat and drink normally and no remains of blood or diarrhoea are found in the faeces.

1: Animals with slightly puffed feathers, movement is slightly less than usual and the faeces may present slight diarrhoea. Respond rapidly to stimuli.

2: Animals with puffed feathers, tend to stay in corners due to hypothermia, although they eat and drink normally. Slightly arched body. May present a little blood in faeces or diarrhoea. Respond rapidly to stimuli although after a while they recover their initial state. Feet and crests are a little pale.

3: Animals stay in corners, have puffed up feathers, feet and crests are pale due to anaemia caused by haemorrhage. An important diminution is observed in regard to the ingestion of food and drink. Their eyes are shut (sleep-like posture) and an arched body. Do not respond rapidly to stimuli and, even if they do, they rapidly recover their initial appearance. They tend to kneel down. Haemorrhagic faeces as well as diarrhoeas may be quite abundant.

4: Death.

Laboratory tests

Nine laboratory studies have been conducted for the assessment of safety of Evanovo and two laboratory studies were conducted for the assessment of the safety of the mixed use of Evanovo together with GUMBOHATCH. Reversion to virulence and test for residual pathogenicity were evaluated in eight laboratory studies, and the safety of one dose and one overdose was evaluated in one study. The special requirements for live vaccines have also been addressed by the applicant.

Safety of the administration of one dose

The safety of the administration of one dose was investigated together with the safety of the administration of an overdose, please refer to the following section.

Safety of one administration of an overdose

One laboratory study was conducted to investigate the safety of administration of a single dose and of an overdose. On day -3 of the study (3 days before hatching), 18-day-old SPF, coccidia-free eggs were administered a 1X dose (group A), a 10X overdose (group C) or PBS (negative control, group B) by in ovo vaccination. Following hatching on day 0, birds were housed under appropriate circumstances to favour reinfection with oocysts. Feed free from anticoccidials and tap water were freely available.

Animals were observed for 21 days with monitoring of clinical signs, mortality, body weight and feed consumption. Oocyst counts were performed during the 21-day follow-up period. On specific study days (which corresponded to the optimal time for scoring of intestinal lesions for each *Eimeria* species) birds from each group were euthanised and intestinal lesions were scored.

Results demonstrated that no clinical signs, mortality or abnormal faeces were observed in the 1X dose group or the 10X dose group.

No statistically significant differences in growth rate or mean weight were detected between groups.

Mean intestinal lesion scores are reported per group per sampling day for duodenum, upper mid-intestine, lower mid-intestine, caeca and rectum, according to the scoring (0 – 4) proposed by Johnson and Reid (1970).

No intestinal lesions with a score greater than 1 were found in any group during the study. The mean lesion score per group for each study day did not exceed 0.33 with no statistically significant differences between groups. Therefore, the study complies with the Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens requirements that no individual score is greater than 3 points, and the average lesion score on each of the sampling day is not greater than 1.5 points.

Concerning *E. praecox*, a statistically significant difference in parasite score (increased number) was reported in the 10X group compared to the control group (but not between the 1X dose group and the control group) at day 4.5, with no differences between any groups at day 14. However, although parasites were detected and higher counts / more birds in 10X group had scores of 1, the higher numbers of parasites did not correlate with any adverse effects (no adverse effect on intestinal function as supported also by no impact on growth of birds in the 10X group), and these findings are consistent with the mechanism of action of the vaccine.

The elimination profile of oocysts and number of oocysts eliminated followed the expected curve for the vaccine and confirmed the administration of a single dose and an overdose. No oocysts were detected in fresh or litter faeces in the control group.

It is accepted that this study demonstrates the safety of the administration of a single dose, and of a 10X overdose in 18-day-old embryonated SPF eggs 3 days before hatching. No adverse reactions were observed following the administration of a single dose, therefore the wording under section 4.6 of the SPC ('None known') is considered acceptable. Although mild coccidial lesions were observed following the administration of a single dose, these lesions did not correlate with clinical signs or any other adverse effects in the single dose group, and such lesions would only (barely) be noticeable if birds were slaughtered shortly after vaccination, which would not occur under field conditions. It can also be accepted that while lesions were slightly more frequent in the 10X overdose group, lesions were as mild as for the 1X dose group, and therefore are not considered necessary to mention in the SPC. Therefore, it is not considered that any additional adverse reactions have been observed following the administration of an overdose and the wording under section 4.10 of the SPC 'No adverse reactions were observed after the administration of a 10-fold overdose.' is considered appropriate.

Safety of the repeated administration of one dose

As Evanovo is intended for single use only in 18-day-old embryonated chicken eggs, the applicant has not conducted any studies to investigate the safety of the repeated administration of one dose. This is considered to be acceptable.

Examination of reproductive performance

Since Evanovo is intended for use in short-lived chickens, the applicant has not conducted any studies to investigate any potential effects on reproductive performance**.** This approach is supported. However, while the applicant suggests that the vaccine is intended for use in broiler chicks, it is noted that the SPC does not restrict use to any particular production type of chicken. The CVMP considers that this is acceptable, given that the vaccine is indicated for single use only in 18-day-old embryonated chicken eggs, and no adverse impact on reproductive performance would be expected taking into account the species-specific affinity to different areas of the intestine. It is not considered that it is necessary to restrict use in the SPC to broilers only, as future layers/breeders could potentially be vaccinated with this vaccine and then at a later stage with a vaccine containing *Eimeria* strains relevant for longer lived birds (i.e., *E. necatrix* and *E. brunetti*). Therefore, by not restricting the vaccine to broilers only (when no restriction is called for on safety grounds), it maintains flexibility for vaccination regimes for poultry farmers.

The applicant has included the words 'Not applicable' under the heading 'Use during pregnancy, lactation or lay' in section 4.7 of the SPC. This is considered acceptable.

Examination of immunological functions

The applicant has not carried out a specific study to examine any adverse effects on immunological function, since, taking into consideration the nature and composition of Evanovo, no negative influence on the immune response is expected. This is considered acceptable taking into account that there are no known immunomodulatory effects associated with live coccidiosis vaccines.

Special requirements for live vaccines

Spread of the vaccine strain

It is a well-known feature of live coccidia vaccines that the vaccinal oocysts spread. The *Eimeria* species included in Evanovo are live attenuated species; the parasites complete their life cycle in the vaccinated animals (triggering the immune response) and, afterwards, they are eliminated through the

faeces to the litter. Then, the oocysts sporulate outside the host and are able to re-infect other in-contact target animals, either previously vaccinated or not. The elimination of oocysts in vaccinated birds was confirmed throughout the safety studies. Spread to non-target species was not investigated because chickens are the only animals that are susceptible to the *Eimeria* species used in Evanovo due to its strong host-specificity.

Dissemination in the vaccinated animal

Dissemination in the target animal was not investigated in a specific study; the trait of each *Eimeria* species relative to their affinity to a specific portion of the intestine is well-recognised and no further studies are considered necessary.

Reversion to virulence of attenuated vaccines

Reversion to virulence and testing for residual pathogenicity were evaluated in eight laboratory studies. Two studies were conducted for each *Eimeria* strain, the first study to conduct five passages in chickens of the master seed parasite (MSP) to obtain passaged material for reversion to virulence testing, the second to test for residual pathogenicity of both the MSP and the passaged MSP. The test for residual pathogenicity for each *Eimeria* strain involved inoculation of at least ten times the number of oocysts that will be included in a standard dose of Evanovo of the MSP or the passaged material (MSP+5) to 14-day-old SPF birds. Mortality, clinical signs, faeces alteration, body weight, oocyst output and species-specific intestinal lesions on the appropriate day post-inoculation were evaluated. Intestinal lesions were also scored on day 14 and at study end on day 21 post-inoculation. The results of the studies demonstrated that for both the MSP and the MSP+5, for each of the three *Eimeria* strains included that are known to induce specific lesions in the intestine (*E. acervulina, E. maxima* and *E. tenella*), the intestinal lesions in the target area were very mild, indicating attenuation of the strain. For *E. praecox*, again there were no differences between the MSP and the MSP+5 groups; parasites were detected in the intestine for a limited period and correlated only with very mild histological changes, indicating that only limited signs of infection were observed. In terms of oocyst output, the pre-patency and patency period, there were no differences between the MSP and corresponding MSP+5 for each of the four *Eimeria* strains. No notable clinical signs, mortalities related to treatment or notable changes in faeces appearance were observed for the MSP or MSP+5 in the data presented for each of the *Eimeria* species included in the vaccine. Overall, it is accepted that the data presented comply with the Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens requirements for the examination of residual pathogenicity of live coccidiosis vaccines, and demonstrate that the vaccine strains are stably attenuated and that no reversion to virulence was observed following five passages *in vivo*.

Biological properties of the vaccine strain

Evanovo contains strains of *Eimeria* that have been attenuated for precocity, the precocious lines were obtained from the virulent parent strains, by serial passages in SPF chickens with the collection of oocysts from faeces within the first few hours after excretion. The biological properties are those of the *Eimeria* species without the pathogenic properties of the wild strains; the vaccine strains have retained the property to stimulate an antigen response but are not associated with adverse clinical signs in the target species and can spread from vaccinated to non-vaccinated animals without reversion to virulence.

Recombination or genomic reassortment of the strains

No specific trials regarding the genomic reassortment or recombination/redistribution of the *Eimeria* strains with other different vaccine strains have been performed. The absence of such studies is considered acceptable; in the event that genomic recombination between one of the vaccine *Eimeria* strains and the respective wild-type *Eimeria* parasite were to occur in the field, the resultant strains would be considered likely to be of equal or less pathogenic characteristics than the parent strains.

User safety

The applicant has presented a user safety risk assessment which has been conducted in accordance with CVMP guideline EMEA/CVMP/IWP/54533/2006.

The main potential routes of accidental contact with the product have been considered and it was concluded that the most likely are those of accidental self-injection, and to a lesser extent dermal or ocular exposure. The vaccine strains are not pathogenic for humans and therefore do not pose a risk for the user.

The excipients are commonly used in other vaccines and do not pose a risk for the user.

The applicant has proposed a standard warning in the SPC and product literature to wash and disinfect hands and equipment after use. This is considered appropriate.

Based on the above risk assessment, it can be concluded that Evanovo does not pose an unacceptable risk to the user when used in accordance with the SPC.

Study of residues

MRLs

No studies on residues have been performed.

The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009.

The excipients listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to 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 when used as in this product.

Consequently, there is no need to perform residue studies.

Withdrawal period

The withdrawal period is set at zero days.

Interactions

Data are provided in support of a proposed compatibility claim use; to support the mixed use of Evanovo with GUMBOHATCH (live vaccine against avian infectious bursal disease registered in the EU via a centralised procedure in 2019) for administration to 18-day-old embryonated chicken eggs. Four laboratory studies have been performed to demonstrate both the safety and efficacy of the associated use, according to the *Guideline on the requirements for combined vaccines and associations of IVMPs* (EMA/CVMP/IWP/594618/2010), which indicates that the basis of association should be a

demonstration of acceptable safety and absence of serious interference between the IVMPs involved. Two of the laboratory studies investigated the safety of the proposed compatibility claim and are assessed below.

One GLP laboratory study was conducted to investigate the safety of administration of a single dose of Evanovo mixed with a single dose of GUMBOHATCH. On day -3 of the study, 18-day-old SPF eggs were administered either the two vaccines mixed (1X maximum dose of each vaccine) (treatment group), or PBS (control group) by in ovo vaccination.

Follow-up was carried out for 35 days after hatching, with monitoring for clinical signs (of both coccidial disease and infectious bursal disease), mortality, body weight and oocyst counts, which were performed during the 35-days follow-up period. On specific study days (which corresponded to the optimal time for scoring of intestinal lesions for each *Eimeria* species) euthanasia and necropsy of randomly selected birds was performed. Intestinal lesion scoring according to Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens was conducted (in addition to microscopic evaluation of infection for parasite numbers and histological changes) and the degree of microscopic bursal damage was scored according to Ph. Eur. 0587 (in addition to evaluation of macroscopic lesions of the bursae, and weight of bursa and spleen).

No clinical signs or changes in faecal appearance were reported in either group. No mortalities were reported related to vaccine administration. Statistically significant differences between groups in bodyweights were reported at different time points, which were not related to vaccination as confirmed in a second study.

Safety parameters related to GUMBOHATCH:

Vaccinated animals showed evidence of significant histological damage to the bursa of Fabricius (BF), with statistically significant differences in lesion score in the BF in the vaccinated group compared to the control group. The mean lesion scores were higher at the first time points but lower mean lesion scores were reported at the last time points, considered as evidence of recovery of the BF by repopulation with lymphocytes after peak bursal damage at days 7 – 14. It was however noted by the CVMP that lesions of score 4 were reported in birds at both day 28 (n = 3, 50% of birds examined) and 35 (n = 2, 33.3% of birds), indicating the presence of severe lesions in birds at these time points. That said, significant bursal lesions were expected to occur in vaccinated SPF birds (with no MDA against IBDV). Ph. Eur. 0587 for avian infectious bursal disease vaccine (live) applies to vaccines containing virus strains of low virulence, thus, GUMBOHATCH does not meet this criterion. This can be accepted, as it has previously been accepted for GUMBOHATCH in the original dossier. It is also noted that birds in the control group had mild bursal lesions. Statistically significant differences in mean histopathological score between groups were detected at all time points, as expected. Additionally, statistically significant differences in the values for mean weights of the bursae of Fabricius and BF:BS ratio; between the vaccinated and control groups were found at all time points. It is noted that section 4.6 of the SPC of GUMBOHATCH states that lymphocyte depletion followed by repopulation and regeneration of the bursa of Fabricius is very common and is not associated with immunosuppression.

The mean body weight was lower in the test group and reflected in statistically significant differences in growth rate between groups. It was raised as a concern that vaccinated animals gained significantly less weight from days 7 – 35 compared to control animals. However, the applicant attributed this difference to the fact that the groups were housed in separate rooms and states that differences in weights between groups attributed to housing factors when using *in vivo* models such as this one is a common occurrence. The applicant concluded therefore that this finding was not a result of associated

vaccine use. This justification was not considered sufficient. Therefore, a new safety study was carried out to confirm this.

Safety parameters related to Evanovo:

Evaluation of intestinal lesions was performed at multiple time points throughout the study. Apart from the macroscopic lesions in two vaccinated birds, no other macroscopic lesions were observed at any of the time points evaluated in the vaccinated group (and no lesions observed in the control group other than a non-specific one). Moderate numbers of parasites were detected following microscopic evaluation of vaccinated birds. No parasites were found in control animal tissues. Microscopic lesions were described in both vaccinated and control animals. Most lesions were mild, and it is proposed by the applicant (supported by a literature reference) that mild lesions in control birds (and indeed a proportion of the vaccinates), particularly in the absence of oocyst shedding in this group, could be attributed to other factors, including diet. Moderate lesions were observed in some of the vaccinated animals, a finding which is consistent with the parasitic scores reported in vaccinated animals and subsequent peak of oocyst numbers in the litter of these animals. These findings, when interpreted in light of the absence of any clinical signs in vaccinated animals, were not considered indicative of a change in the safety profile of Evanovo due to associated use with GUMBOHATCH and would appear to be generally consistent with the findings presented in the laboratory safety study for Evanovo in the current dossier, however no formal comparison has been presented by the applicant.

Oocyst elimination profiles are supportive of vaccinal *Eimeria* strains replicating *in vivo* post-vaccination and appeared to be similar to when Evanovo is used alone (based on the data provided in the laboratory studies provided in the dossier).

The CVMP considered that based on the results obtained in this safety study, the overall safety profile of Evanovo with respect to intestinal lesions and oocyst elimination profiles did not appear to be notably affected by mixed use with GUMBOHATCH; however, considering the differences in weight gain, the applicant provided an additional laboratory safety study. The study was conducted to assess the impact of the associated administration of GUMBOHATCH and Evanovo when mixed prior to in ovo administration to 18-day-old embryonated chicken eggs on individual body weight. On day -3 of the study, 18-day-old SPF eggs were administered either the two vaccines mixed (single maximum dose of GUMBOHATCH and Evanovo,), Evanovo only or GUMBOHATCH only. Following hatching, birds were weighed and each treatment group was divided into four groups that were balanced for baseline study weight and were housed together in a single cage. All cages were kept in the same room, under identical conditions in order to avoid any potential effect of housing conditions on daily weight gain. Measures were taken to avoid contamination between groups during the study.

Follow-up was carried out for 35 days after hatching, with monitoring for clinical signs (of both coccidial disease and infectious bursal disease), mortality, body weight and oocyst counts.

The results demonstrated that no statistically significant differences in body weight or growth rates were observed between treatment groups during the study.

No clinical signs or mortalities vaccine-related were observed in the three groups during the study.

Oocyst elimination profiles are supportive of vaccinal Eimeria strains replicating *in vivo* post-vaccination. No oocysts were detected in samples obtained from the GUMBOHATCH group, indicating that no cross-contamination with Eimeria species occurred in this group.

The CVMP accepts that the data presented support the safety of mixed use of Evanovo and GUMBOHATCH. No adverse impact on weight gain or growth rates up to 35 days of age, when compared to separate use of either Evanovo or GUMBOHATCH, was observed following mixed use. In this study, all birds were housed in the same room and thus were exposed to identical conditions. This

is in contrast to the previous laboratory study, in which treatment groups were housed separately and during which differences in weight gain and growth rate were observed.

IBDV seroconversion of 100% of birds in the GUMBOHATCH + Evanovo and GUMBOHATCH groups by study day 35 was an expected outcome as a result of vaccination. One bird in the Evanovo group was also found to be seropositive for IBDV antibodies at day 35. This indicates that cross-contamination with IBDV between groups occurred during the study as a result of all birds being housed in the same room. Indeed, the study was designed specifically to remove any possible effect of housing conditions on weight gain, and, as such, the potential for cross contamination could not be entirely removed, despite measures that were put in place to minimise this risk. It is noted however that this minimal cross-contamination did not affect weight gain of the birds in the Evanovo group. It is therefore accepted by the CVMP that this finding in one bird does not impact on the overall interpretation of the study results, specifically that the mixed use of GUMBOHATCH with Evanovo does not have a negative effect on weight gain or growth rates up to 35 days of age, when compared to separate use of either Evanovo or GUMBOHATCH only.

The combined results of both laboratory safety studies are considered to adequately support the conclusion that the associated use of Evanovo and GUMBOHATCH mixed together before administration to embryonated chicken eggs can be considered as safe as when either GUMBOHATCH or Evanovo are administered separately.

In conclusion, the safety of the proposed compatible use claim for mixed administration of Evanovo and GUMBOHATCH is considered to have been sufficiently supported.

In addition to the study provided above, with respect to other interactions, the applicant claims that no known interactions between live vaccines against coccidia and other vaccines commonly administered to chickens have ever been reported and that chances are low that interaction would be of relevance when the product is used as indicated. In addition, the applicant refers to the fact that in the field trial, animals were vaccinated in accordance with established vaccination programs for each farm. Thus, the applicant claims that this vaccine would not interfere with vaccinations commonly administered to chickens.

Furthermore, the *Eimeria* species included in Evanovo are sensitive to the most frequently used anticoccidials and to the other agents having anti-coccidial activity. Thus, an incompatibility statement on this point has also been included in section 4.8 of the SPC to warn against use of anticoccidial drugs via feed or water for at least 3 weeks following vaccination. Additionally, given that the duration of immunity is dependent on continuous recycling of oocysts in the environment in which vaccinated birds will be housed, a warning is also included that a decision to use anticoccidial substances in the period after 3 weeks post-vaccination should be made taking into account the potential impact on the duration of immunity. An appropriate warning is included to state that no anticoccidial substances or other agents having anticoccidial activity via feed or water should be used for at least 3 weeks following the hatching of eggs vaccinated with this product otherwise the correct replication of the vaccine oocysts, and consequently the development of a solid immunity, could be hindered.

Finally, the standard statement is included to state that no information is available on the safety and efficacy of this immunological veterinary medicinal product when used with any other veterinary medicinal product except the product mentioned above (i.e., GUMBOHATCH).

Field studies

One GCP compliant, multicentre, randomised, double blind, double-dummy positive-controlled clinical field trial was conducted to evaluate the safety and efficacy of Evanovo under field conditions. The study was conducted in three commercial broiler farms in one EU member state (Belgium) stated to be

representative of management and conditions for standard broiler production in the EU, with historical records of clinical or subclinical coccidiosis. Whilst it is normally expected that clinical field studies should be conducted in more than one geographical region, given that different sites (albeit within the one country) have been included, the CVMP is prepared to accept that the data generated may be considered sufficiently representative of field conditions in the EU. A double-dummy study design was used to account for the fact that the positive control is administered by coarse spray at one day of age, with the use of an appropriate placebo, both for in-ovo vaccination of the positive control group, and for coarse spray of the test group at one day of age. A total of 219,996 18-day-old embryonated broiler eggs were randomly assigned to two treatment groups and received either the test product (Evanovo, n=108,471) or placebo (PBS, for the group of eggs which will be vaccinated with the positive control at one day of age, n=112,525). Following hatching, at one day of age, chicks which had been vaccinated in ovo with Evanovo received placebo (the solvent of a commercially available vaccine against coccidiosis, without adjuvant, n=103,092) or the positive control (a commercially available vaccine against coccidiosis, n=104,728), by the oral route (coarse spray).

During the study, on each farm the test and control groups were housed in separate housing units, both with identical handling conditions. Animal housing and management was conducted according to the common rearing practices. Standard feed free of coccidiostats and water were freely available. No drugs to which *Eimeria* could be sensitive were administered during the study. All study animals were vaccinated against Gumboro, Newcastle disease and avian infectious bronchitis during the study period.

Animals underwent a follow-up period of 41 – 44 days after vaccination, the approximate period of fattening in broiler chicks in the EU. During this time, daily monitoring of adverse reactions, clinical signs, faeces appearance and mortality was conducted. Euthanasia and intestinal lesion scoring was performed on 15 animals in each group on days 7, 22, 28 and 35 on each farm. Body weight, feed consumption and oocyst output in fresh faeces and in litter samples were also evaluated.

The study was well conducted and confirmed that the product is safe for use in 18-day-old embryonated broiler chicken eggs under field conditions. There were no statistically significant differences in hatching rate between groups. No adverse effects, clinical signs related to vaccination or changes in faeces appearance were observed in either the test or control group. Although a statistically significant difference in body weight between groups was reported on day 0, this was considered to be clinically irrelevant, and no statistically significant differences in mean body weight were observed between groups at day 22. The overall mean mortality rate in both groups was similar and no statistically significant differences were reported. The mean intestinal lesion index at day 7 post-vaccination was very low in the positive control group and nil in the test group. Mean intestinal lesion index on study day 22 was lower in the EVANOVO group than in the positive control group, which was statistically significantly different, however it is noted that this is not likely to be clinically relevant given the overall low mean score for intestinal lesion index score.

Other parameters evaluated in the study as efficacy variables can also be considered supportive of safety; feed conversion rate, oocyst counts, intestinal lesions on days 28 and 35, and body weight at the end of the study, for which it was demonstrated that there were no differences between treatment groups.

Environmental risk assessment

An environmental risk assessment, conducted in accordance with the requirements of the 'Environmental risk assessment for immunological veterinary medicinal products' (EMA/CVMP/074/95), was provided.

Considerations for the environmental risk assessment

It is widely described that the parasites of the genus *Eimeria* are characterised by their rigid and strong host specificity. Not only are the *Eimeria* species naturally limited to a narrow range of host species, but also to a specific site of infection in the intestine. The potential hazard of the vaccine strains included in Evanovo to be transmitted to non-target species is effectively nil.

Vaccinated chickens are expected to shed oocysts of each *Eimeria* species included in the vaccine, however this does not involve any risk to non-vaccinated target animals since the vaccine strains are attenuated and data are provided that demonstrate the absence of risk of reversion to virulence. Shedding of the *Eimeria* strains by vaccinated animals is an expected event and is important for re-infection and maintenance of immunity.

Genetic recombination of the strains included in Evanovo with field strains is possible, but the resultant strains would be expected to be of equal or less pathogenic characteristics than the parent strains, that is, if an attenuated strain were to recombine with a field strain it is accepted as being unlikely to result in a strain with increased pathogenicity.

Apart from the antigen, the rest of the vaccine components are well-known excipients widely used in pharmaceutical formulations. They are generally regarded as nontoxic at these low concentrations used.

It is not considered that there is any likelihood of the live attenuated vaccinal parasites to cause hazards to the environment, taking into account that use as recommended ensures that all the resuspended vaccine is injected into embryonated chicken eggs, and no parasites are released into the environment. If transmission of vaccine parasites to non-vaccinated chicks occurs, clinical signs of disease will not occur due to the attenuation of the vaccine strains. Transmission to non-target species, including humans, is not expected.

Based on the data provided the environmental risk assessment can stop at Phase I. Evanovo is not expected to pose a risk for the environment when used according to the SPC. No specific control measures are needed in addition to the general management recommendations of poultry farms and the standard precautions included in the package leaflet concerning the handling and disposal of unused veterinary medicinal product or waste materials derived from the use of such product.

Overall conclusions on the safety documentation

Nine GLP-compliant laboratory trials and one multicentric GCP-compliant field trial were carried out to assess the safety of Evanovo. In addition, two laboratory trials which investigated the safety of associated use were carried out, one of which was GLP-compliant. The safety of vaccination has been evaluated in the intended sub-category of target species; 18-day-old embryonated chicken eggs, in laboratory studies using SPF eggs and in the field study using commercial broiler eggs.

The laboratory studies were conducted in compliance with the requirements of Ph. Eur. 2326 (Coccidiosis vaccine (live) for chickens) for the investigation of safety, with the exception of the route of administration of the vaccine, this deviation is acceptable given that the only route of administration proposed for this vaccine is in ovo.

During the safety studies, clinical signs, mortality, weight, faeces appearance, oocyst production and intestinal lesion scores were monitored. The scoring of intestinal lesions followed the system described by Johnson and Reid (1970), incorporated in Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens, for the species-specific lesions visible in the intestine for *E. acervulina*, *E. maxima* and *E. tenella*. For *E.*

praecox, which is known not to induce macroscopic lesions, the intestinal tract was examined for the presence of parasites and histological changes.

One laboratory study was conducted to investigate the safety of administration of a single dose and of an overdose. Given that Evanovo is intended for single use only, the safety of the administration of a repeat dose was not necessary to investigate. No clinical signs, mortalities, abnormal faeces or changes in the appearance of birds were reported in either the 1X dose group or the 10X dose group. No statistically significant differences in growth rate or mean weight were detected between groups. The scoring of intestinal lesions demonstrated that no more than mild coccidial lesions were observed in the single dose group and in the 10X dose group. The study complies with the Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens requirements that no individual score is greater than 3 points, and the average lesion score on each of the sampling day is not greater than 1.5 points. The elimination profile of oocysts and number of oocysts eliminated followed the expected curve for the vaccine and confirmed the administration of a single dose and an overdose.

Since Evanovo is intended for use in short-lived chickens, it is considered acceptable that the applicant has not conducted any studies to investigate any potential effects on reproductive performance. It is noted that there is no text proposed in the SPC to restrict use to any particular production type of chicken. The CVMP considers that this is acceptable, given that the vaccine is indicated for single use only by the in ovo route, and no adverse impact on reproductive performance would be expected.

It is accepted that no specific investigations have been performed to examine any adverse effects on immunological function, since no negative influence on the immune response is expected due to vaccination with Evanovo.

The special requirements for live vaccines were satisfactorily addressed for Evanovo, as discussed below.

Concerning spread of the vaccine strain, it is a well-known feature of live coccidia vaccines that the vaccinal oocysts spread. The elimination of oocysts in vaccinated birds was confirmed throughout the safety studies. Spread to non-target species was not investigated because chickens are the only animals that are susceptible to the *Eimeria* species used in the vaccine due to its strong host-specificity.

Dissemination in the target animal was not investigated in a specific study; the trait of each *Eimeria* species relative to their affinity to a specific portion of the intestine is well-recognised and no further studies are considered necessary.

Reversion to virulence and testing for residual pathogenicity were evaluated in compliance with the Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens requirements and demonstrate that the vaccine strains are stably attenuated and that no reversion to virulence was observed after five passages *in vivo*.

The biological properties of the vaccine strains have been sufficiently described; the vaccine *Eimeria* strains have been attenuated for precocity which enables the strains to retain the ability to stimulate an antigen response but are not associated with adverse clinical signs in the target species, and can spread from vaccinated to non-vaccinated animals without reversion to virulence.

It is accepted that no specific trials regarding the genomic reassortment or recombination of the *Eimeria* vaccine strains with other different *Eimeria* strains have been performed. In the event that genomic recombination between one of the vaccine *Eimeria* strains and the respective wild-type *Eimeria* parasite were to occur in the field, it is accepted that the resultant strains would be considered likely to be of equal or less pathogenic characteristics than the parent strains, given that the vaccinal strains are attenuated.

It is accepted that the use of Evanovo does not pose a risk to the user when used in accordance with recommendations given in the product information. The instructions to 'Wash and disinfect hands and equipment after use' is considered sufficient for inclusion in the SPC section 4.5.

The proposed withdrawal period of zero days is considered acceptable.

Concerning interactions, the applicant has proposed a compatibility use claim for mixed use of Evanovo and another vaccine for in ovo use, GUMBOHATCH. Two laboratory studies were presented which supported that the safety profile of GUMBOHATCH when used alone and the safety profile of Evanovo when used alone is not adversely affected by mixed use. The safety of mixed use is considered to have been adequately supported by the data presented.

One multicentre, randomised, double blind, double-dummy positive-controlled clinical field trial, conducted in accordance with GCP guidelines, was performed to investigate the safety and efficacy of Evanovo under field conditions. The data demonstrated the safety of vaccination under field conditions of use in 18-day-old embryonated chicken eggs. No adverse reactions or clinical signs attributable to vaccination were observed in the study, the mortality rate was low and within the expected range for the farms included. No intestinal lesions were observed in the test group at day 7 post-vaccination, the expected peak time for potential vaccine-related intestinal lesions. Efficacy variables (e.g. body weight, feed conversion rate) also demonstrated that there were no differences for these parameters between groups and therefore can be accepted as being indirectly supportive of the safety of Evanovo under field conditions. The field study confirmed the safety of administration of EVANOVO when used in accordance with recommendations; administration by in ovo to 18-day-old embryonated chicken eggs.

Evanovo is not expected to pose a risk for the environment when used according to the SPC. Standard disposal statements are included in the product literature.

Part 4 – Efficacy

Introduction and general requirements

The vaccine is intended for the active immunisation of chickens to reduce clinical signs, intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria acervulina*, *Eimeria maxima, Eimeria praecox* and *Eimeria tenella*. A single dose of the vaccine is intended for in ovo use in 18-day-old embryonated chicken eggs, following dilution in the solvent (sterile PBS) provided for use with the vaccine ('HIPRAHATCH'). The vaccine can be diluted in the solvent to achieve a final dose volume for use in automated egg injection machines of 0.05 ml or 0.10 ml per egg (the dose of vaccine administered is unchanged, it is only the volume of the solvent which can vary).

The onset of immunity is claimed as 21 days of age and the duration of immunity is proposed as 63 days of age in an environment that permits oocysts recycling.

Efficacy was demonstrated in compliance with the European Directive 2001/82/EC (as amended by 2004/28/EC and Directive 2009/9/EC), and the European Pharmacopoeia (Ph. Eur.) chapter 5.2.7 as well as Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens.

Generally, seven species of *Eimeria* that affect chickens are internationally accepted to be the causative agents of avian coccidiosis, a disease which is distributed worldwide and causes substantial economic losses throughout the world. These species are: *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. necatrix*, *E. tenella*, *E. praecox* and *E. mitis*. The pathogenicity of the first five strains is widely recognised since they can lead to clinical coccidiosis in which the affected birds generally show typical symptoms of the disease, such as bloody droppings and increased mortality. *E. necatrix* and *E. brunetti* mainly affect birds of more than 8 – 9 weeks of age and thus, they are not needed in vaccines

intended for use in chickens that are slaughtered at an early age. The other two *Eimeria* species which can affect chickens, *E. praecox* and *E. mitis*, are known to cause subclinical coccidiosis, as the affected birds show no clear symptoms of the disease; however, this subclinical form may lead to uncontrollable bodyweight and uniformity (out of standard). The repercussions on the productive performances are due to its early impact in the cycle, it is especially important in broiler meat productions where the first weeks are crucial for the overall performance of the bird. Thus, four species were considered as essential to be included as active ingredients in Evanovo: *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella*. The CVMP considers that the inclusion of the four selected *Eimeria* strains for Evanovo are adequately justified considering the intended target species, short-lived chickens.

Challenge model:

No specific studies for the development of the challenge model for each *Eimeria* strain were presented; the applicant claims that the pathogenicity of these species is well-documented. In addition, Evanovo is the fourth coccidiosis vaccine developed by the applicant, and previous experience regarding coccidiosis, including challenge models, has been applied when developing this new vaccine. The *Eimeria* species used to perform the challenge were heterologous from the strains included in the vaccine. The vaccinal strains are Spanish field isolates whereas the challenge strains were isolated in the UK at the coccidia reference laboratory of Houghton Poultry Research Station in Compton (UK). The challenge strains were inoculated by oral-gavage because it is the normal infection route for the coccidiosis disease model, the results obtained in the trials fully demonstrate the suitability of the challenge strains and the dose chosen.

Considering that the challenge studies were conducted in accordance with the immunogenicity requirements of Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens, it is accepted that the challenge models used for each *Eimeria* species were appropriate.

Efficacy parameters and tests:

The efficacy parameters as provided in Ph. Eur. 2326 Coccidiosis vaccine live for chickens; oocyst production, intestinal lesions, signs of disease and growth rate were investigated in the efficacy studies. The parameters chosen are considered appropriate for evaluating the efficacy of the product.

Efficacy documentation

Two GLP-compliant laboratory tests and one combined GCP-compliant field safety and efficacy study have been performed for the assessment of the efficacy of Evanovo. In addition, a GLP standard laboratory efficacy study was provided in support of the mixed use of Evanovo with another vaccine (GUMBOHATCH), and a GLP-compliant laboratory efficacy study in support of the efficacy of GUMBOHATCH, following mixed use with Evanovo.

Laboratory studies were well documented and carried out in 18-day-old embryonated eggs, as recommended for vaccination. Recycling of oocysts is an important factor for the efficacy of live coccidiosis vaccines. In line with the Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens, following hatching the animals included in the laboratory trials were housed in suitable floor pens to favour reinfection with vaccine oocysts. Under field conditions, commercial farms were enrolled and thus, standard housing conditions were used.

Two batches of Evanovo were used in the efficacy trials and were produced at the same manufacturing facilities that will be used for future production batches, in accordance with the proposed manufacturing process. Vaccine batches, which are used to test efficacy, are required to contain the

most attenuated passage to be used for production. According to the manufacturing method for Evanovo, the passage level of the antigen for production is a fixed value from the Master Seed parasite; there is no range.

The investigation of the efficacy of the vaccine was performed at the minimum content of sporulated oocysts that will be present in a dose.

Laboratory trials

Two GLP laboratory studies were performed to investigate the efficacy of Evanovo alone, one to investigate the onset of immunity (OOI) and one to investigate the duration of immunity (DOI), discussed in the sections below, in addition to two laboratory studies to investigate the efficacy of Evanovo mixed with GUMBOHATCH, and the efficacy of GUMBOHATCH when mixed with Evanovo (both of these studies are summarised under 'Interactions').

Onset of immunity

The onset of immunity was evaluated in one study, in which at 18 days of embryonation (study Day -3), one group of eggs was vaccinated in ovo with a minimum dose of Evanovo (group A) and one group of eggs was mock-vaccinated with PBS (group B). During the vaccination period, vaccinated and control eggs and hatched chicks were handled identically; after hatching (study Day 0) each group was allocated in a separate room, however, both rooms were handled identically (birds in each group were maintained under the same housing conditions at the same density, on solid floors with shavings or similar covering to favour reinfection with oocysts). At 21 days of age, animals were randomly distributed in 4 groups and four separate challenge sub-studies were conducted for each *Eimeria* species, with vaccinated chickens and mock-vaccinated control chickens in each challenge study. After each *Eimeria* challenge, chickens were observed at least daily for 14 days post-challenge. During this period, chickens were allocated in cages. All operations during the challenge period were performed on a blinded basis. Feed free from anticoccidials and tap water were freely available. All operations during the challenge period were performed blinded to treatment.

Clinical signs, faeces appearance, mortality, body weight and oocyst counts were evaluated during the 14 day observation period. Intestinal lesions were evaluated at the time at which lesions were expected to be most severe for the three *Eimeria* strains known to be associated with characteristic intestinal lesions; *E. acervulina, E. maxima* and *E. tenella*. Intestinal lesions were also evaluated for *E. praecox* on an appropriate day post-challenge, in addition to histological analysis and evaluation of number of parasites present in the intestine. Lesions were scored in the remaining birds in each group on day 14. Intestinal lesions were evaluated using the scoring system outlined in Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens for *E. acervulina*, *E. maxima* and *E. tenella*. For *E. praecox*, this species is considered not to induce macroscopic lesions (Ph. Eur. 2326 requires that microscopic evidence of infection is evaluated to compare differences between vaccinated and control groups, based on the count of number of parasites in a section of tissue and histological changes due to infection), however the applicant did evaluate macroscopic intestinal lesions (based on an in-house developed scoring system on a three point scale) in addition to microscopic evidence of infection The assessment of appearance of faeces of a group of poultry was performed using the following scale: 0; normal, 1; less than 25% of faeces are affected, 2; between 25-50% of faeces are affected, 3; between 50 – 75% of faeces are affected, 4; more than 75% of faeces are affected. The clinical signs of poultry were assessed by evaluating the general state of health of the group and also the appearance of faeces, with the same scoring system for clinical signs of coccidiosis as used in the safety studies.

In each challenge study, the study complied with the requirements of Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens for establishment of infection in the control group (intestinal lesions with a score not less than 2 in ≥80% control birds) and protection in the vaccinated group (≥80% vaccinated birds have no or minimal lesions in the intestine, e.g. mean lesion scores not greater than 1, no bird has a score of 4).

Intestinal lesions were statistically significantly different (reduced) in the vaccinated group compared to the control group for each *Eimeria* challenge study, as indicated below (mean lesion scores indicated in parenthesis):

- *E. acervulina*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.33) and control group (2.00).

- *E. maxima*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.67) and control group (2.22).

- *E. praecox*: a statistically significant difference ($p<0.001$) was observed between the vaccinated (0.72) and control group (1.61) for macroscopic changes (according to the applicant's in-house scoring system for macroscopic lesions). A statistically significant difference (reduction) in parasite score ($p<0.001$) (mean score of 0.83 and 2.00 in the vaccinated and control group, respectively) and in histological changes ($p<0.001$) (mean score of 0.56 and 1.06 in the vaccinated and control group, respectively) were observed following microscopic evaluation of intestinal lesions.

- *E. tenella*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.39) and control (2.83) group.

Oocyst production was decreased in the vaccinated group compared to the control group, with a percentage reduction of 99.8%, 81.9%, 93.5% and 91.2% for *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella*, respectively. Although it is noted that oocyst output was not statistically compared between groups, it can be accepted that the data support a reduction in oocysts output considering that the percentage reduction was very high (between 81.9% – 99.8%) in the vaccinated group compared to the control group for each of the *Eimeria* challenges, therefore it is considered that the proposed claim for a reduction of oocyst output has been adequately demonstrated.

Clinical signs evaluation demonstrated that for each of the challenge studies, mild to moderate changes in faeces were observed in the control group of each respective study (scores 1 and 2, exceptionally score 3) for the *E. acervulina*, *E. maxima* and *E. praecox* challenges and that mild to moderate changes in faeces (score 1 and 2) were observed in the vaccinated group in these challenge studies. The duration of changes in faeces in the vaccinated group compared to the control group was also similar. For *E. tenella*, more severe changes in faeces appearance were induced in the control group, in addition to mortalities. In the *E. tenella* vaccinated group, a similar duration of changes in faeces was reported, with changes of mild to moderate severity. With respect to clinical signs, the *E. tenella* challenge was the only capable of inducing sufficient clinical signs in the control group to allow a meaningful comparison of clinical signs of disease between groups. Thus, the applicant provided further justification in support of the claim for a reduction of clinical signs of disease. The applicant addressed this issue by presenting a general score per group and a comparison of the % average daily prevalence of clinical signs between groups.

The approach was accepted by CVMP.

For each *Eimeria* challenge, conducted at 21 days of age, statistically significant differences in the % average daily prevalence between the vaccinated and control groups were reported. However, based on reviewing the raw data of the study, including the scoring of clinical signs for individual birds, and the faeces appearance of each of the three cages per study group, it is considered that the differences

in scores between groups were attributed to the incidence of diarrhoea, with the exception of the *E. tenella* challenge. Therefore, the data are considered inadequate for the purposes of supporting a broader claim for a reduction of clinical signs, other than diarrhoea, with the exception of *E. tenella*, for which it was noted that more severe signs of disease, including death, were observed in the post-challenge period, and therefore the statistically significant reduction of clinical signs for this strain can be accepted as having been demonstrated.

Finally, regarding growth rate, while a claim for a positive effect of vaccination to reduce infection-related growth impairment is not sought for this vaccine, the Ph. Eur. monograph 2326 Coccidiosis vaccine (live) for chickens requires that the growth rate is significantly greater in the vaccinates than in the controls. A statistically significant difference in growth rate was observed in each of the challenge studies, during the immediate post-challenge phase for *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella,* supporting that growth rate was greater in the vaccinated group during the acute challenge period. However, the growth rate in the control group quickly recovered after the challenge, and a statistically significant difference in overall growth rate between day – 1 to 14 post-challenge was observed only in the *E. acervulina* challenge study. It is accepted that compliance with the Ph. Eur. monograph 2326 Coccidiosis vaccine (live) for chickens requirement that the growth rate in the vaccinated group is statistically significantly greater than in the control group was demonstrated, at the time when the growth rate was most affected by challenge.

In summary, it is accepted that the study supports a reduction in intestinal lesions and a reduction in oocyst output for each of the *Eimeria* strains included in Evanovo and that the onset of immunity of 21 days of age is supported following in ovo vaccination of 18-day-old embryonated SPF chicken eggs for these parameters. Regarding the claimed indication for reduction of clinical signs, CVMP considered it appropriate to restrict this claim with the word diarrhoea in parenthesis for *E. acervulina*, *E. maxima* and *E. praecox* in the absence of other clinical signs of disease, whereas the broader claim for a reduction of clinical signs for *E. tenella* was considered to have been adequately supported, given that more severe clinical signs of disease, including mortality, were induced following challenge, with a statistically significant reduction in clinical signs in the vaccinated group.

Duration of immunity

One study was carried out in the target species to investigate the duration of immunity. At 18 days of embryonation (study Day -3), one group of eggs was vaccinated in ovo with a minimum dose of Evanovo (group A) and one group of eggs was mock-vaccinated with PBS (group B). At 63 days of age, animals were randomly distributed in 4 groups and four separate challenge sub-studies were conducted for each *Eimeria* species, with vaccinated chickens and mock-vaccinated control chickens in each challenge study. The study designs and parameters measured were similar to the onset of immunity study, with evaluation of clinical signs, faeces appearance, mortality, body weight and oocyst counts during the 14 day observation period.

Intestinal lesions were statistically significantly different (reduced) in the vaccinated group compared to the control group for each of the four *Eimeria* strains included in the vaccine. There were no indications of any decrease in the level of protection in vaccinated birds at 63 days of age in an environment which permitted recycling of oocysts.

- *E. acervulina*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (mean score of 0.25) and control (mean score of 2.92) groups.
- *E. maxima*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.25) and control (2.50) group.

- *E. praecox*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated and control group, based on a scoring system from 0 – 2 (mean lesion score was 0 in the vaccinated group and 1.25 in the control group). Following microscopic evaluation of infection, a statistically significant difference in parasite score ($p<0.001$) (reduction) and a statistically significant difference (reduction) in histological changes were reported in the vaccinated group compared to the control group; the mean scores for parasites were 0 and 1.83 in the vaccinated and control group, respectively, and the mean scores for histological changes were 0 and 1.50 in the vaccinated and control group, respectively. Whilst 12/12 birds in the control group had parasites detected in the area examined (10 – 100 parasites, n=2, >100 parasites; n=10) <10 parasites (score 0) was reported in all 12 birds in the vaccinated group. Similarly, mild to moderate histological changes were observed in all control birds but were absent in the vaccinated group.

- *E. tenella*: a statistically significant difference ($p<0.001$) was found in intestinal lesion scores between the vaccinated (0.17) and control (3.75) groups.

Oocyst production was decreased in the vaccinated group compared to the control group, with a percentage reduction of oocyst excretion in the vaccinated group compared to the control group during day 3 to 14 post-challenge of 97.6%, 92.2%, 99.7% and 99.2% for *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella*, respectively.

Clinical signs consisting of mild to moderate changes in faeces appearance were observed in the vaccinated groups for each of the challenge studies (maximum score 2), and mild to moderate changes (score 2, exceptionally score 3) were also observed in the control groups (*E. acervulina* and *E. praecox*) or more severe changes with maximum scores of 3 or 4 on some of the days *(E. maxima, E. tenella*). However, as discussed for the study conducted to investigate the OOI, no conclusions could be drawn regarding any potential differences in clinical signs between groups. The applicant addressed this issue by presenting a general score per group and a comparison of the % average daily prevalence of clinical signs between groups. For each *Eimeria* challenge, conducted at 63 days of age, statistically significant differences in the % average daily prevalence between the vaccinated and control groups were reported, as follows:

- *E. acervulina*; the % average daily prevalence was 0.56 and 13.06 in the vaccinated and control group, respectively ($p<0.001$).
- *E. maxima*; the % average daily prevalence was 1.39 and 23.61 in the vaccinated and control group, respectively ($p<0.001$).
- *E. praecox*; the % average daily prevalence was 1.11 and 5.83 in the vaccinated and control group, respectively ($p<0.003$).
- *E. tenella*; the % average daily prevalence was 3.33 and 47.22 in the vaccinated and control group, respectively ($p<0.001$).

However, similar to the data presented at onset of immunity, upon review of the raw data it was noted that the differences were attributed to the incidence of diarrhoea, therefore the data are considered inadequate for supporting a broader claim for a reduction of clinical signs other than diarrhoea, again with the exception of *E. tenella*, for which more severe signs of disease manifested following challenge.

The weight of the vaccinated and control groups was comparable on the day prior to challenge for each sub-study. A statistically significant decrease in growth rate was observed in the control group in the immediate post-challenge period following *E. acervulina*, *E. maxima* and *E. tenella* challenge or in the case of *E. praecox* slightly later (between days 4.5 to 14 for *E. praecox*). However, with the exception

of *E. praecox*, there was no statistically significant differences between groups for the overall growth rate due to compensatory growth in the control group.

In summary, it is accepted that the study supports a reduction in intestinal lesions and a reduction in oocyst output for each of the *Eimeria* strains included in Evanovo and that the duration of immunity of 63 days of age is supported following in-ovo vaccination of 18-day-old embryonated SPF chicken eggs for these parameters. Similar to the conclusion reached regarding the OOI challenges, it is accepted that a statistically significant reduction in clinical signs has been observed for each of the four challenges at duration of immunity, but that for *E. acervulina*, *E. maxima* and *E. praecox*, the clinical signs that were induced by the challenges were limited to changes in faeces only and thus the indication was restricted to a reduction of clinical signs (diarrhoea) for these *Eimeria* strains. For *E. tenella*, the broader claim for a reduction of clinical signs was considered to have been adequately supported, given that more severe clinical signs of disease were induced following challenge, with a statistically significant reduction in clinical signs in the vaccinated group.

Maternally derived antibodies (MDA)

Whilst Evanovo is proposed for in ovo use in 18-day-old embryonated chicken eggs (3 days prior to hatch), no product-specific studies were conducted to determine if the presence of MDA would interfere with the response to vaccination, however this approach has been justified by the applicant on the basis of a) the nature of the immunological response triggered after an *Eimeria* infection; both humoral and cell-mediated, however the cell-mediated response is likely to play a major role in protection against avian coccidiosis, b) the fact that vaccines with either virulent or attenuated strains have been used since 1950 worldwide, in the majority of cases, chicks vaccinated with live vaccines come from breeders that have been previously vaccinated also with live vaccines, without specific indication of lack of efficacy in such cases, and c) data provided for the applicant's predecessor vaccines which were considered demonstrative of the absence of interference of MDAs with the response to vaccination. In addition, during the application procedure for the centralised product EVALON, it was accepted by CVMP that the presence of MDA does not impact the development of immunity against *Eimeria* species. The CVMP considers that this conclusion can reasonably be extrapolated to Evanovo, considering the similarities between the vaccines. The same information was presented in the dossier for EVANT and was accepted as demonstrative of a lack of negative impact of MDA on the response to vaccination.

In summary, it is accepted that the presence of maternally derived antibodies would not be expected to have a negative impact on vaccination with Evanovo.

Interactions

One GLP laboratory study is presented, in which the efficacy of mixed use of one dose of Evanovo and GUMBOHATCH in 18-day-old embryonated chicken eggs was investigated. GUMBOHATCH is a commercially available vaccine for vaccination against avian infectious bursal disease virus and was authorised by the centralised route for use in chickens (either 18-day-old embryonated chicken eggs, in ovo use, or 1-day-old broiler chickens, subcutaneous route).

At 18 days of embryonation (study Day -3), one group of SPF chicken eggs was vaccinated in ovo with a dose of the association of Evanovo + GUMBOHATCH vaccines (group A), and a second group of eggs was mock-vaccinated with PBS and used as control (group B). The dose of each vaccine was the minimum dose and at the most attenuated passage level. During the vaccination period, vaccinated and control eggs and hatched chicks were handled identically and housed as for the laboratory studies presented previously. Before challenge, animals were sampled for blood in order to check antibody levels against IBDV, to confirm the correct vaccination with GUMBOHATCH and to demonstrate that the

control group remained seronegative. At 21 days of age, animals were randomly distributed in 4 groups and four separate challenge studies were conducted for each *Eimeria* species, with vaccinated chickens and mock-vaccinated control chickens in each challenge. For each *Eimeria* challenge, chickens were observed at least daily for 14 days post-challenge. There was no IBDV challenge in the study.

Follow-up consisted of evaluation of intestinal lesions, growth rate, clinical signs and oocysts counts (identical to the onset of immunity and duration of immunity studies).

The results demonstrated that a statistically significant difference (reduction) of intestinal lesions in the Evanovo + GUMBOHATCH group compared to the mock-vaccinated negative control group in accordance with the requirements of Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens for *E. acervulina* (mean lesion scores of 0 vs 2.00 in group A and B, respectively), *E. maxima* (mean lesion scores of 0.78 vs 2.22 in group A and B, respectively) and *E. tenella* (mean lesion scores of 0.56 vs 2.83 in group A and B, respectively). At least 80% of the vaccinated birds had no or minimal lesions in the intestine (e.g. mean lesion scores not greater than 1) and no bird had a lesion score of 4, when marked characteristic lesions were observed in not fewer than 80% of the control group animals (lesion scores not less than 2). For *E. praecox*, a significant reduction in lesions based on the internal scoring system was reported in the vaccinated group (mean score of 0.44 and 1.61 in the group A and B, respectively), and a significant reduction in the microscopic evidence of infection in the intestine (parasites score and histological changes score) was demonstrated in accordance with the requirements of Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens.

Oocyst production was decreased in the Evanovo + GUMBOHATCH group compared to the control group, with a percentage reduction of oocyst excretion in the vaccinated group compared to the control group during day 3 to 14 post-challenge of 98.0%, 87.3%, 96.2% and 92.4% for *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella*, respectively.

Growth rate in the vaccinates was statistically significantly greater in the Evanovo + GUMBOHATCH vaccinated group than in the controls during the acute post-challenge period, as for the OOI and DOI studies. However, apart from the *E. acervulina* challenge in which a statistically significant difference in growth rate in favour of the vaccinated group was reported, no statistically significant differences in growth rate between groups during the overall follow-up period were reported, based on compensatory growth in the control group (again, similar to the OOI and DOI studies). Regarding clinical signs, the data were similar to the efficacy data following use of Evanovo alone and were not considered to support a claim for a reduction of clinical signs of disease, however in response to questions, the applicant presented a comparison of the % average daily prevalence between groups, as for the OOI and DOI of Evanovo when used alone, and, for each *Eimeria* challenge, conducted at 21 days of age following vaccination in ovo with Evanovo mixed with GUMBOHATCH, statistically significant differences in the % average daily prevalence between the vaccinated and control groups were reported, as follows:

- *E. acervulina*; the % average daily prevalence was 0.56 and 6.94 in the vaccinated and control group, respectively ($p<0.001$).
- *E. maxima*; the % average daily prevalence was 1.67 and 5.00 in the vaccinated and control group, respectively ($p=0.016$).
- *E. praecox*; the % average daily prevalence was 1.94 and 5.28 in the vaccinated and control group, respectively ($p=0.029$).
- *E. tenella*; the % average daily prevalence was 8.33 and 25.50 in the vaccinated and control group, respectively ($p<0.001$).

Therefore, the data presented are consistent with that presented at onset of immunity when Evanovo is used alone, therefore, it can be accepted that the protection for this parameter is unaffected following mixed use with GUMBOHATCH.

Overall, it is accepted that the use of Evanovo mixed with GUMBOHATCH does not appear to negatively affect the efficacy of Evanovo (onset of immunity), as evaluated by challenge at 21 days of age. Given that the onset of immunity is not affected following mixed use, it can be accepted that there is no evidence of interference and, consequently, it is reasonable to assume that the duration of immunity will not be subject to interference.

Concerning GUMBOHATCH, anti-IBDV antibody titres for each group demonstrated that antibodies against IBDV were present in all vaccinated animals and the control group remained negative for IBDV antibodies. The applicant was requested to provide appropriate data to support that the efficacy of GUMBOHATCH will not be negatively impacted by mixed use with Evanovo.

In response to questions raised, the applicant provided an additional laboratory challenge study to investigate the efficacy of GUMBOHATCH when administered mixed with Evanovo. This study was a GLP-compliant, randomised, blinded laboratory challenge study conducted to investigate the efficacy of GUMBOHATCH, when administered mixed with Evanovo, by challenge with a very virulent IBDV challenge strain. The OOI at 24 days of age (i.e., the currently authorised onset of immunity for GUMBOHATCH) following in ovo vaccination was investigated in 18-day-old embryonated seropositive chicken eggs. Two groups of animals were used, one vaccinated with Evanovo+GUMBOHATCH in ovo (group A) and one control mock-vaccinated group (group B). On day -3, both vaccines were administered mixed in a dose at minimum titre.

At 24 days of age, animals from groups A and B were challenged with a vv IBDV strain, by the oculonasal route, (the same challenge strain and route of administration as used to support the efficacy of GUMBOHATCH). Chicks were observed daily for 6 days after challenge, with monitoring of clinical signs (with a scoring system used for clinical signs relevant to IBDV infection, as per the original efficacy studies conducted for GUMBOHATCH), mortality, serological response and weight of animals. At 6 days post-challenge, when the acute phase of the infection was expected, animals were necropsied and bursa of Fabricius (BF) and spleen were weighed and examined macroscopically. Bursae were examined macroscopically for presence of external oedema, followed by histopathological analysis. Growth rate of animals and the oocysts eliminated were also monitored.

No clinical signs were observed in either group in the pre-challenge period from hatching (day 0) to challenge (day 24). After challenge, no clinical signs or mortality were observed in group A. No mortality occurred in group B, however 4 birds displayed mild clinical signs compatible with Gumboro disease. The relevance of the mild clinical signs in group B was demonstrated by a corresponding statistically significant difference in growth rate between day 23 to day 30 of the study; mean growth rate of 531.9 g in group A compared to 330.1 g in group B ($p<0.001$). The differences between the study groups for the proportion of clinical signs were statistically significantly different. Nevertheless, the fact remains that no clinical signs were observed to occur in the vaccinated birds.

External oedema of the bursa was observed in 90% of animals in group B and in no bursae of group A ($p<0.001$).

There was a statistically significant difference observed between groups for the sum of macroscopic lesions of the bursa of Fabricius at day 30; mean score 0.40 in the vaccinated group vs 3.50 in the control group ($p<0.001$).

Histopathological examination of bursal lesions was carried out. The summary score for 'acute histological functional lesions score', demonstrated a statistically significant difference between group A (2.80) compared to group B (13.0), $p<0.001$. It is accepted that the histopathological scoring system used in this study is appropriate and allows the differentiation between vaccine-induced bursal damage and vvIBDV-induced bursal damage. This is also consistent with the approach taken to support the indications for use in the original dossier for GUMBOHATCH.

A statistically significant difference between groups A and B for BF:BW ratio was observed; group A; 0.821, group B; 2.154 ($p<0.001$), which the applicant claims is characteristic of the acute phase of a vvIBDV infection in the control group and reflects the severe inflammation that took place in the bursa. A statistically significantly higher spleen: BW ratio, indicative of splenomegaly in group B (1.663) was observed compared to group A (0.852) ($p<0.001$).

Concerning the serological response, MDAs were present in chicks, decreasing on day 23 (day before challenge). However, on day 30, a serological response in group A was evident, whilst the control group B remained with low titres despite the challenge 6 days previously.

The oocyst elimination profile demonstrated oocyst elimination and recycling in the vaccinated group. No oocysts were detected in the control group faeces.

It is concluded that this study is supportive of the currently authorised indications for use of GUMBOHATCH, when this vaccine is administered mixed with Evanovo. Whilst a study group vaccinated with GUMBOHATCH alone was not included in this study, this is acceptable considering that a mock-vaccinated control group was included, and that the results following challenge of the vaccinated group (GUMBOHATCH plus Evanovo) supported the currently authorised indications for use at the onset of immunity time point, that is, a reduction in clinical signs and lesions of the bursa of Fabricius caused by very virulent IBDV. The study was conducted in commercial broiler eggs with MDA against IBDV, consistent with the fact that GUMBOHATCH should only be used in chickens with MDAs against IBDV and it is noted that the average ELISA units of the hatchability control group was within the range stated in the product information for the level of MDAs at hatching for which efficacy of the vaccine has been demonstrated.

Overall, it is accepted that the efficacy of GUMBOHATCH is not negatively affected by the simultaneous administration (mixed use) of Evanovo when administered in ovo at the 18th day of embryonation, at the onset of immunity time point of 24 days of age. Although this study only investigated efficacy by way of challenge with vvIBDV strain at the approved onset of immunity (24 days) and no data has been provided in respect of challenge at the approved duration of immunity (up to 45 days), given the findings of this study and the absence of any negative impact on efficacy at the onset of immunity and therefore lack of demonstrated interference of combined use of Evanovo and GUMBOHATCH, there is no reason to believe that similar findings would not also be expected 21 days later at the approved duration of immunity for this product.

In summary, it is accepted that adequate data have been provided to support the efficacy of the associated use of Evanovo mixed with GUMBOHATCH, and that mixed use will not be expected to have any impact on the efficacy of either vaccine, compared to that which may be expected when either vaccine is used alone.

Field trials

One GCP compliant, multicentre, randomised, double blind, double-dummy positive-controlled clinical field trial was conducted to evaluate the safety and efficacy of Evanovo under field conditions. The efficacy parameters measured were compared between the test group and a positive control group (vaccinated with a commercially available vaccine against coccidiosis at 1 day of age). The control product (a commercially available vaccine against coccidiosis) can be accepted as being suitable given that it includes the same *Eimeria* species included in Evanovo (*E. acervulina, E. maxima, E. praecox* and *E. tenella*) in addition to another.

The primary efficacy variable was the feed conversion rate (FCR), calculated on each farm by dividing the total amount of feed consumed by each group by the body weight of each group at slaughter. Secondary efficacy variables consisted of evaluation of oocyst counts, macroscopic intestinal lesions on

day 28 and 35, body weight, and mortality and faeces appearance (included as safety and efficacy variables).

The results demonstrated that, following vaccination of 18-day-old commercial broiler chicken eggs with Evanovo (n=103,092) or the positive control vaccine (n=104,728) at one day of age by coarse spray, no difference in the FCR was found between the test (FCR 1.57 ± 0.042) and control group. In the absence of a negative control group, and notwithstanding that the FCR was not statistically significantly different between groups when comparing the mean, any conclusions that may be drawn from the comparison of the FCR between groups is limited.

Secondary efficacy variables also appear to generally support comparability between the Evanovo group and the positive control group; there were no differences in weight of chickens in the test and control groups during the study or at time of slaughter, and the mortality rate was similar and within expected ranges for the farms included. The evaluation of intestinal lesions and oocyst outputs in faeces confirmed vaccine take and replication of *Eimeria*, for both the test group and positive control group.

The mean intestinal lesion index on study day 28 was very similar between groups and was the same on day 35. Differences at both time points were not statistically significant between groups. It is noted that the mean score was attributed to evaluation of several intestinal regions, and lesions were largely absent in regions other than the duodenum and upper mid-intestine, which resulted in a low mean score. Intestinal lesion index was also reported according to intestinal region on days 28 and 35. There were no differences between groups for mean scores for each intestinal region, other than a non-clinically relevant in the test group. At day 28, lesion scores of 1 were very common in both the test and control group (with one score of 2 reported in each group), while at day 35, lesions scores of 1 and 2 were reported in animals of both the test and control group (on one farm only) whereas lesions ≤1 were reported in animals of both groups on the other two farms. These data would seem to suggest a degree of infection pressure on this farm compared to the other two farms. However, as for the other farms, it can be accepted that intestinal lesion scores were comparable between the test and positive control group.

Oocyst elimination profiles showed that oocyst counts were higher in the positive control group than the test group. Notwithstanding this point, the profiles were generally similar between groups. In this field study, the oocyst kinetics appeared to be generally similar to that observed in the laboratory safety study in the 1x dose group. Oocyst counts declined in litter faeces in both the study groups on days 28, 35, and 42, which the applicant considers corresponds to the onset of cellular immunity and cessation of the cycling of vaccinal oocysts, in addition to demonstrating protective immunity against natural infection. However, in the absence of a negative control group, such a hypothesis cannot be confirmed as it is unknown to what extent field strains were prevalent in the rearing units of the farms concerned during this production cycle.

Whilst it is noted that it is possible that there was infection pressure given the manner in which the three commercial broiler farms were stated to have been selected, it is not possible to confirm if a natural outbreak occurred, in the absence of a negative control group. While the rationale for not including a negative control group is noted, the absence of a negative control group limits the conclusions that may be made as it is unknown, for example, if broiler chicken performance was improved by vaccination. That said, FCR results of previous batches in the farms participating in the study was provided. In order to provide more reassurance that the farms were subject to infection/infestation pressure, the applicant was requested to provide supportive documentation for each of the three farms concerning the history of clinical/sub-clinical coccidiosis on each farm, and to detail what vaccination schedules were employed on the farms in the previous two production runs. The applicant provided this information. Furthermore, further details were provided concerning the

selection process of the farms, together with stated confirmation of the veterinarians involved in the study that records of previous clinical coccidiosis exist, and confirmation that at each of the locations in the field study, oocyst numbers were recorded on or after study day 28. It is considered that sufficient information has been provided to support that the farms included in the field trial were subject to adequate infection pressure.

Overall, it can be accepted that the Evanovo group appears to have performed comparably to the positive control group for the efficacy variables evaluated (feed conversion rate, weight, mortality, oocyst production demonstrating replication of vaccinal strains, intestinal lesions). However, given the absence of a negative control group, the study data can be considered as supportive only regarding the efficacy of Evanovo. That said, the findings from this study are considered supportive of the efficacy findings from the OOI and DOI challenge studies.

Overall conclusion on efficacy

Two GLP standard laboratory tests and one combined GCP standard field safety and efficacy study have been presented in support of the claimed efficacy of Evanovo. In addition, two GLP standard laboratory efficacy studies were provided in support of the mixed use of Evanovo with another vaccine, GUMBOHATCH. In all studies, the vaccine was administered in accordance with recommendations (in ovo vaccination, to 18-day-old embryonated chicken eggs).

The results from the laboratory studies conducted to investigate the OOI and the DOI at the minimum dose according to the proposed range on the label for each *Eimeria* species demonstrate that the product has been shown to be efficacious for the active immunisation of chicks when vaccinated in ovo at 18-days of embryonation against coccidiosis caused by *E. acervulina*, *E. maxima*, *E. praecox* and *E. tenella* to reduce intestinal lesions, clinical signs (diarrhoea) and oocyst output. In addition, adequate data was provided to support a reduction of clinical signs for *E. tenella.*

Compliance with the immunogenicity requirements of Ph. Eur. 2326 Coccidiosis vaccine (live) for chickens were demonstrated at both onset and duration of immunity (i.e., validity of challenge and level of protection demonstrated in vaccinated birds).

The OOI and the DOI are accepted as 21 days and 63 days of age, respectively, when animals are vaccinated in an environment which permits oocyst recycling.

The presence of MDA in the target species are not expected to interfere with the response to vaccination.

A compatibility claim for associated use of Evanovo with another authorised vaccine for in ovo use, GUMBOHATCH, a vaccine against avian infectious bursal disease virus, also indicated for in ovo use is proposed. It is accepted that the efficacy studies provided in support of this proposal demonstrated an absence of interference (no negative effect) on the efficacy of Evanovo when challenged against the four *Eimeria* strains at 21 days of age following mixed use with GUMBOHATCH, and an absence of interference (no negative effect) on the efficacy of GUMBOHATCH when challenged against vvIBDV at 24 days of age following mixed use with Evanovo. Thus, the proposed mixed use of Evanovo together with GUMBOHATCH is considered to have been satisfactorily supported.

One multicentre, randomised, positively-controlled, double-dummy field study was conducted to investigate the safety and efficacy of Evanovo under field conditions. Following vaccination of 18-day-old embryonated chicken eggs from commercial broiler farms with Evanovo, the feed conversion rate (primary efficacy variable) was stated to be non-inferior in the Evanovo group compared to the positive control group vaccinated with a commercially available live avian coccidiosis vaccine. The mean FCR between groups was not reported as being statistically significantly different between

groups and was stated to be non-inferior in the test group compared to the positive control group. Secondary efficacy variables (weight, mortality, oocyst production demonstrating replication of vaccinal strains, intestinal lesions) supported comparability between the test and control groups. Although it is noted that it is possible that there was adequate infection pressure in the three commercial broiler farms included in the trial, it is not possible to confirm if a natural outbreak occurred, in the absence of a negative control group. While the rationale for not including a negative control group is noted, the absence of a negative control group limits the conclusions that may be made. Thus, the field data can be considered as supportive only in terms of concluding on the efficacy of Evanovo.

Overall, it can be accepted that the data provided demonstrate the efficacy of Evanovo at 21 and 63 days of age, following in ovo vaccination, for the active immunisation against coccidiosis caused by *E. acervulina, E. maxima, E. praecox* and *E. tenella* to reduce intestinal lesions and oocyst output. However, the proposed broader claim for a reduction of clinical signs of disease, both at onset and duration of immunity, was not considered to have been adequately supported and the final wording is as follows:

"For the active immunisation of chickens to reduce clinical signs (diarrhoea), intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria acervulina*, *Eimeria maxima* and *Eimeria praecox*, and for the reduction of clinical signs, intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria tenella*.".

Part 5 – Benefit-risk assessment

Introduction

Evanovo is a live coccidiosis vaccine containing 4 different attenuated species of *Eimeria*, proposed for the active immunisation of chickens against coccidiosis caused by *Eimeria acervulina*, *Eimeria maxima*, *Eimeria praecox* and *Eimeria tenella* to reduce clinical signs of disease, intestinal lesions and oocysts output, following in ovo vaccination of 18-day-old embryonated chicken eggs.

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

Benefit assessment

Direct therapeutic benefit

The *Eimeria* strains included in Evanovo are claimed to be the four most important Eimeria strains which affect broiler chickens currently in the EU. While the applicant also holds a marketing authorisation for a vaccine which is authorised by the centralised procedure and which has the same strains (and more) as included in Evanovo, the route of administration is different for Evanovo (in ovo) which is intended to facilitate automated vaccination at hatcheries in large numbers, compared to administration by coarse spray in 1 day old chickens.

The data provided demonstrate that Evanovo is efficacious for the active immunisation of chickens to reduce clinical signs (diarrhoea), intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria acervulina*, *Eimeria maxima* and *Eimeria praecox*, and for the reduction of clinical signs, intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria tenella*. The onset of immunity was demonstrated at 21 days of age, and the duration of immunity was demonstrated at 63 days of age, at both time points in accordance with the immunogenicity requirements of the Ph. Eur. monograph 2326, which outlines the level of protection required to be established for a live coccidiosis vaccine for use in chickens.

Additional benefits

Evanovo is administered by in ovo vaccination to chicken eggs at 18 days of embryonation (3 days prior to hatching). This method of administration is becoming increasingly popular for poultry vaccines.

Evanovo increases the range of available treatment possibilities and routes of administration for live, attenuated coccidiosis vaccines for chickens.

The increase of vaccines available for the reduction of coccidiosis would have an indirect benefit on reducing the use of anticoccidial agents.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

Safety:

Risks for the target animal:

The safety of Evanovo was investigated in accordance with requirements, including taking into account the special requirements for live vaccines. During the safety studies, clinical signs, mortality, weight, faeces appearance, oocyst production and intestinal lesions were monitored. The scoring of intestinal lesions followed the system described by Johnson and Reid (1970), incorporated in Ph. Eur. 2326, Coccidiosis vaccine (live) for chickens for the species-specific lesions visible in the intestine for *E. acervulina*, *E. maxima* and *E. tenella*. For *E. praecox*, which is known not to induce macroscopic lesions, the intestinal tract was examined for the presence of parasites and histological changes. The safety data provided for Evanovo demonstrate that for the target animal, there were no adverse reactions following vaccination under recommended conditions of use. The vaccine strains have been attenuated for precocity, by selection of oocysts with shorter pre-patent periods, which enables the vaccine strains to maintain immunogenic properties without causing disease. However, as live sporulated oocysts (the infective form of the parasite) are included in Evanovo, the main risk to the target species is that of the vaccine strains reverting to a virulent form. In order to investigate this risk, the applicant has presented well-conducted laboratory studies which collectively demonstrated that the vaccine strains did not revert to a virulent form after five *in vivo* passages in chickens.

Concerning spread of the vaccine strain, it is a well-known feature of live coccidiosis vaccines that the vaccine oocysts spread. The elimination of oocysts in vaccinated birds was confirmed throughout the safety studies. Spread to non-target species was not investigated because chickens are the only animals that are susceptible to the *Eimeria* species used in Evanovo due to strong host-specificity.

Risk for the user:

The use of Evanovo does not pose a risk to the user when used in accordance with recommendations given in the product information. The instructions to 'Wash and disinfect hands and equipment after use' is considered appropriate for inclusion in the SPC section 4.5.

Risk for the environment:

The *Eimeria* oocysts in the vaccine Evanovo will be shed by chickens after vaccination and will be present in the environment where chickens are maintained. However, the recycling of oocysts is a

desired attribute of the vaccine, necessary for the development of immunity, and the risk to the environment of the presence of the attenuated vaccine oocyst strains is considered to be acceptable. The attenuated vaccine strains are highly host specific and do not cause disease in the target species. In addition, the presence of coccidia is considered to be ubiquitous in commercial poultry farms and the risk to the environment would not be expected to be any greater than the risk presented by the naturally present coccidia species. Thus, the risks for the environment following use of the vaccine as recommended are considered to be negligible.

Evanovo is not expected to pose a risk for the environment when used according to the SPC recommendations. Standard advice on waste disposal is included in the SPC.

Risk for the consumer:

There are no risks identified for consumers of animals vaccinated with Evanovo. The active substances included in Evanovo are live attenuated *Eimeria* strains which do not infect humans. The active substance being a principle of biological origin intended to produce active immunity is not within the scope of Regulation (EC) No 470/2009. In addition, excipients listed in section 6.1 of the SPC are either allowed substances for which table 1 of the annex to 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 when used as in this product, therefore a withdrawal period is not required.

Risk management or mitigation measures

No specific risk management or mitigation measures are considered necessary for Evanovo. General measures are included in the product literature to ensure safe and efficacious use of the vaccine. There is a recommendation included that litter should be removed and facilities and material cleaned between production cycles. In addition, in order to allow development of immunity, warnings are included in the SPC to ensure that chickens are strictly floor-reared in the first three weeks of life (to allow recycling of oocysts), and that no anticoccidial substances or agents having anticoccidial activity are used for at least three weeks after hatching. In addition, given that maintenance of immunity is dependent on continuous recycling of oocyst, a warning that the DOI is dependent on recycling of oocysts is also included.

Evaluation of the benefit-risk balance

At the time of submission, the applicant applied for the following indication:

For the active immunisation of chickens to reduce clinical signs, intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria acervulina, Eimeria maxima, Eimeria praecox* and *Eimeria tenella*.

The product has been shown to be efficacious for the proposed indications, with the exception that for *E. acervulina*, *E. maxima* and *E. praecox*, the reduction of clinical signs was considered to be related to a reduction of diarrhoea only, and the CVMP agreed to the following indication(s):

For the active immunisation of chickens to reduce clinical signs (diarrhoea), intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria acervulina, Eimeria maxima* and *Eimeria praecox,* and for the reduction of clinical signs, intestinal lesions and oocysts output associated with coccidiosis caused by *Eimeria tenella*.

Information on development, manufacture and control of the active substance and finished product has been presented and lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. It is well tolerated by the target animals and presents an acceptable risk

for users and the environment when used as recommended. Appropriate precautionary measures have been included in the SPC and other product information.

Based on the data presented, the overall benefit-risk is considered positive.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for Evanovo 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.