21 March 2019
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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use

CVMP assessment report for Baycox Iron (EMEA/V/C/004794/0000)

International non-proprietary name: toltrazuril / iron(iii) ion

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

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Introduction

The applicant Bayer Animal Health GmbH submitted on 24 November 2017 an application for a marketing authorisation to the European Medicines Agency (The Agency) for Baycox Iron, through the centralised procedure under Article 3(2)(a) of Regulation (EC) No 726/2004 (optional scope).

The eligibility to the centralised procedure was agreed upon by the CVMP on 12 April 2017 as Baycox Iron contains a new fixed combination of two active substances (toltrazuril and iron) which was not authorised as a veterinary medicinal product in the Union on the date of entry into force of Regulation (EC) No 726/2004.

The active substances in Baycox Iron are toltrazuril, an antiprotozoal substance, and iron (as gleptoferron), an anti-anaemic substance. The target species is piglets (pigs).

Baycox Iron is a suspension for injection for piglets containing 36.4 mg/ml toltrazuril and 182 mg/ml iron (as gleptoferron). The product is available in multi-dose glass vials containing 100 ml.

The applicant applied for the following indication: "For the prevention of clinical signs of coccidiosis in neonatal piglets on farms with a confirmed history of coccidiosis caused by *Cystoisospora suis* and concurrent prevention of iron deficiency anaemia in piglets."

The rapporteur appointed is Gerrit Johan Schefferlie and the co-rapporteur is Ewa Augustynowicz.

The dossier has been submitted in line with the requirements for submissions under Article 13b of Directive 2001/82/EC – a fixed combination application.

On 21 March 2019, the CVMP adopted an opinion and CVMP assessment report.

On 20 May 2019, the European Commission adopted a Commission Decision granting the marketing authorisation for Baycox Iron.

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 5.0, dated 2 July 2018) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided, the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the active substance takes place at sites located both inside and outside the EU. Valid GMP certificates for all sites have been issued by EU competent authorities and QP declarations, based

on on-site audits by the manufacturing site responsible for batch release have been provided.

Manufacture of the dosage form, primary and secondary packaging and batch control take place at a site within the EU which is covered by a manufacturing authorisation issued by a competent authority. Batch release takes place at Produlab Pharma B.V., Raamsdonksveer (The Netherlands) which holds a manufacturing authorisation issued by the competent authority of the Netherlands.

Valid GMP certificates were provided by the applicant for all the sites concerned. The GMP certificates can also be found in the Eudra GMDP database.

Overall conclusions on administrative particulars

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

The GMP status of all the active substance and finished product manufacturing sites has been satisfactorily established and is in line with legal requirements.

Part 2 - Quality

Composition

The finished product is presented as an aqueous suspension for injection containing 3.64% w/v toltrazuril and 18.2% w/v iron (as gleptoferron) as the active substances.

Other ingredients are: sodium chloride, polysorbate 20 and polysorbate 80, phenol and water for injections.

The product is presented in multi-dose glass injection vials containing 100 ml.

Containers

The primary packaging consists of a colourless silicon-coated glass Type II vial closed with a red chlorobutyl rubber stopper and an aluminium cap. The materials comply with the relevant Ph. Eur. and/or EU requirements.

The glass vials are depyrogenated and sterilised prior to filling.

The chlorobutyl rubber stoppers are pre-sterilised by gamma-irradiation.

The choice of the container-closure system, and the gamma-irradiation sterilisation dose range used for the stoppers, have been validated by stability data and are adequate for the intended use of the product.

The secondary packaging is a cardboard box, each box containing 1 vial of 100 ml.

Development pharmaceutics

Active substances

Toltrazuril:

Toltrazuril has polymorphic forms, but only the stable form is manufactured for this product. Toltrazuril is insoluble in water. To produce homogenous and low viscous suspensions toltrazuril must be micronized. Therefore, the final particle size and polymorphic form are both critical.

Gleptoferron:

This is a macromolecular complex of partially hydrated β-ferric oxyhydroxide and glucoheptonic acid. The active substance can exist only if associated with excipients as an aqueous solution, it is therefore purchased as an intermediate product.

Formulation

A multi-dose formulation containing a combination of toltrazuril and the gleptoferron complex was the aim of the development. The physico-chemical properties of both active substances dictate that an injectable suspension would be the necessary formulation type.

Before development started, a quality target product profile (QTPP) and (critical) quality attributes ((c)QA's) of this type of formulation were defined to provide the boundary conditions for formulation development.

Calculations regarding the feasible doses of iron and toltrazuril and the feasible injection volumes yielded a composition range of 18.2% w/v of active iron and 0.91 to 3.64% w/v of toltrazuril for the formulation. Siliconized glass vials of Type II glass with a nominal volume of 100 ml and red chlorobutyl rubber stoppers were selected as the primary packaging materials.

After completion of the clinical dose finding, the final injectable suspension formulation chosen was the one containing iron (III) 18.2% w/v (as gleptoferron) plus toltrazuril 3.64% w/v. Phenol was selected as the preservative since all EU marketed iron dextran injectable solutions contain phenol and its inclusion is therefore justified.

Compatibility with the primary packaging materials was demonstrated. An overfill to 103 ml was justified to ensure withdrawal of at least the nominal fill volume of 100 ml out of a vial.

The final formulation and the selected packaging materials met all the quality criteria and quality attributes which had been defined in the Quality Target Product Profile (QTPP). The suspension obtained is easy to redisperse, easy to administer as either a subcutaneous or intramuscular injection, and deemed suitable for the treatment of the target animals.

The formulation used during the clinical studies is exactly the same as that intended for marketing.

Production

An aseptic production process is required for this injectable suspension.

The critical production steps and parameters were identified and justified in-process control steps were included. The finished product can be produced reliably according to its specification with the proposed manufacturing process.

The maximum number of punctures of the rubber closure has been justified. Section 4.9 of the SPC includes recommendations for the use of draw-off needles or an automatic dosing device, as appropriate.

Method of manufacture

The bulk suspension is aseptically filled into the pre-sterilised and depyrogenated 100 ml glass vials on an automated filling line. The vials are then closed with the rubber stoppers and sealed with the aluminium caps.

The in-process controls are adequate for this type of manufacturing process.

Except for the toltrazuril (sterile) and phenol, the intermediate GleptoMed 20% Fe and the excipients

sodium chloride, polysorbate 20 and polysorbate 80 and water for injections are each tested for microbiological purity prior to use. The microbiological quality of the ingredients used in the manufacture of the product is acceptable. An in-process control for bioburden prior to filtration is considered unnecessary considering the absence of any microbiological contamination found due to the preservative efficacy of both the solutions used to manufacture the product. The choice of sterilising filter has been explained and sufficient validation data have been provided. The sterilisation of toltrazuril by gamma-irradiation has been justified. The pre-sterilisation of the vials and the stoppers is justified.

The choice of a non-terminal sterilisation process is justified according to the Annex to the Note for guidance: Development pharmaceutics for veterinary medicinal products: Decision trees for the selection of sterilisation methods (EMEA/CVMP/065/99) and is acceptable.

The manufacturing process is considered to be a non-standard manufacturing process. It has already been satisfactorily validated using four batches of at least the commercial batch size.

Control of starting materials

Active substances

Iron (as gleptoferron 20% Fe)

Gleptoferron is regarded as the active substance and it contains iron (III) as the active moiety.

Gleptoferron is solely manufactured as an intermediate product (gleptoferron 20% Fe = GleptoMed 20% Fe) which contains the excipients water and phenol (in addition to the gleptoferron). There is no clear distinction between the manufacture of the active substance and that of the intermediate product.

Toltrazuril

The chemical name of toltrazuril is 1-methyl-3-[3-methyl-4-[4-(trifluoromethyl)thio]phenoxy]-phenyl] 1,3,5-triazine-2,4,6-(1H,3H,5H)-trione.

Toltrazuril is a crystalline powder, soluble in ethyl acetate, and practically insoluble in water.

Toltrazuril is not hygroscopic.

Toltrazuril has an achiral molecular structure.

Polymorphism has been observed for this active substance.

The physico-chemical characteristics liable to affect toltrazuril's bioavailability are it's particle size and polymorphic form. In addition to the test for its melting point, a second test, using X-ray powder diffractometry, for the produced crystalline form is included in the specifications for the active substance. Therefore, it is ensured that toltrazuril is consistently released as correct polymorphic form. All the information on the active substance is provided within the dossier. The two active substance manufacturers produce the active substance in the same manner resulting in toltrazuril of identical quality

The characterisation of the active substance is in accordance with the Guideline on the chemistry of new active substances (EMEA/CVMP/QWP/707366/2017). Defined potential and actual impurities were sufficiently well discussed with regards to their origin and adequately characterised.

Information on the manufacture of the active substance has been provided in the dossier.

The quality of water used in the last steps of the synthesis is not guaranteed to contain endotoxin levels below 0.25 EU/mg. However, after its sterilisation the toltrazuril is tested at the manufacturer of

the finished product for sterility and endotoxins on a routine basis. Sufficient in-process controls are applied during its synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented and are satisfactory.

There is no monograph for toltrazuril in the Ph. Eur. and an in-house monograph is defined. The active substance specification is acceptable and includes tests for appearance, identity (IR, melting point [Ph. Eur.] and polymorphism)), colour/clarity of solution (Ph. Eur.), sulphate (Ph. Eur.), heavy metals (Ph. Eur.), loss on drying (Ph. Eur.) assay and impurities (HPLC). An additional test for polymorphic form (XRPD) is also included. The same specification is applicable to gamma-irradiated toltrazuril with an additional test for sterility.

All impurities were limited within the qualification threshold according to VICH GL10.

The analytical methods used have been sufficiently described and the non-compendial HPLC method appropriately validated in accordance with VICH GL2.

Satisfactory information regarding the reference standards used for the assay and impurities testing has been presented.

Batch analysis data for six batches (three full scale production batches from each manufacturing site) of the active substance have been provided. The results are well within the specifications and consistent from batch to batch.

Stability data for three production scale batches of toltrazuril manufactured at the one site, stored in HDPE bags for 60 months at 25 °C/60% RH, 60 months at 30 °C/80% RH and 6 months at 40 °C/75% RH, were provided. Stability data were also provided from one scale-up batch and the three production scale batches of toltrazuril manufactured at the other site, stored in HDPE bags for 12 months at 25 °C/60% RH, 12 months at 30 °C/75% RH and 6 months at 40 °C/75% RH, and these substantiate the claimed 24 months retest period.

In addition, stability data were provided from three commercial batches of gamma-irradiated toltrazuril stored in PE bags for 36 months at 25 °C/60% RH, 36 months at 30 °C/75% RH and 6 months at 40 °C/75% RH.

The humidity of the intermediate storage conditions was higher than the VICH requirement, but was acceptable as it was more challenging.

The following parameters were tested: appearance, identity, melting point, clarity and colour of solution, loss on drying, specified impurities, single and total unspecified impurities, total impurities and assay. The analytical methods used were in accordance with the active substance specifications and were stability indicating.

Photostability testing following the VICH guideline GL5 was performed on one batch.

The applicant has suitably justified that after more than 20 years the degradation pathway is well known. It is therefore acceptable that no further stress testing data are provided.

All tested parameters were well within specification and no real trends in physical characteristics, assay or impurity profiles were observed. The stability results indicate that toltrazuril is very stable and not light sensitive. The proposed retest period of 24 months is acceptable. The stability results of gamma-irradiated (sterilised) toltrazuril demonstrate that gamma-irradiation does not affect the chemical and physical characteristics of the active substance.

Excipients

All excipients are well known pharmaceutical ingredients and their quality is compliant with their

respective current Ph. Eur. monographs.

There are no novel excipients used in the finished product formulation.

The list of excipients is included in section 6.1 of the SPC.

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

The product does not contain any materials derived from human or animal origin.

Valid TSE declarations from the manufacturers of the finished product have been provided.

Confirmation has been provided that no materials of animal origin are used in the manufacture of the stoppers for the finished product packaging.

Control tests during production

Iron (as gleptoferron 20% Fe) could not be classified as an active ingredient product only as it contains the excipients water and phenol in addition to the gleptoferron. As explained above there is no clear distinction between the manufacture of the active substance, gleptoferron complex, and that of the intermediate product (GleptoMed 20% Fe = Gleptoferron 20% Fe), as the manufacture takes place in an aqueous environment from the start.

The gleptoferron complex is a complex of iron (III) hydroxide oxide dextranglucoheptanoate hydrate. Its complete structure is not available. The dossier enclosed an acceptable bibliographical proposal for the iron-dextran complex structure (London, E., J. of Pharmaceutical Sciences, vol. 95, nº 7, July 2004).

The molecular formula of gleptoferron is x FeH_2O.n $C_6H_{10}O_5$. $C_7H_{14}O_8$. y H_2O.

Iron and the polysaccharide dextran are the most important raw materials in gleptoferron.

There is no clear distinction between the manufacture of the active substance and that of the intermediate product, as the manufacture takes place in an aqueous environment from the start, therefore the documentation belonging to parts II.C and II.D cannot be clearly separated. The complete documentation is provided to justify the quality of both the active substance (gleptoferron) and the intermediate (GleptoMed 20% Fe).

The glucose units and the α-linkages cause the chirality of gleptoferron. The resulting value of the specific optical rotation of the dextran chains is not measurable because of the strong inherent colour of the iron (III) hydroxide oxide dextranglucoheptanoate hydrate complex at a wave length of 589.3 nm.

Regarding the physico-chemical characteristics liable to affect bioavailability, gleptoferron forms a colloidal solution in the finished product, so the possibility of polymorphism of the active substance is not relevant.

There is no monograph for gleptoferron in the Ph. Eur. and an in-house specification has therefore been defined.

The proposed specification for gleptoferron includes tests for identity (iron, dextran), purity (chloride), pH, non-volatile residue, relative density, kinematic viscosity content of iron (atomic absorption spectometry), and content of dextran (TOC). All the methods are Ph. Eur. test methods.

The specification proposed for the intermediate GleptoMed 20% Fe is adequate. Test procedures are described in detail. The test methods are all described in the Ph. Eur. and as a consequence their

validation is not required.

Adequate in-process controls are applied during the synthesis/complexation/intermediate manufacture. The specifications and control methods for intermediate products, starting materials and reagents have been presented and are satisfactory.

Detailed information on the manufacture of gleptoferron has been provided. The active substance is synthesised in 3 main steps using well-defined starting materials. The starting material specifications are acceptable. Potential and actual impurities were discussed with regards to their origin and have been adequately characterised.

Detailed information on the manufacture of the intermediate, GleptoMed 20% Fe, has been provided. The manufacturing process consists of two main steps: preservation and adjusting the pH and content of the solution, then filtration and filling.

Stability data from 6 batches of gleptoferron 20% Fe, from the proposed manufacturer and stored in the intended commercial package under long term conditions (24 months at 25 °C/60% RH and 36 months at 25 °C/40% RH), intermediate conditions (12 months at 30 ºC/65% RH) and accelerated conditions (6 months at 40 °C/75% RH and 40 °C/NMT 25% RH) according to the VICH guidelines, have been provided. Photostability testing was performed in accordance with VICH GL5.

The 12-month holding time proposed for the intermediate GleptoMed 20% Fe has been adequately justified.

Control tests on the finished product

The proposed finished product specification is applicable for use both at release and at the end of shelf life and is appropriate to control the quality of the finished product. The finished product specification includes tests for appearance, redispersibility, identification of the iron, toltrazuril (UV, HPLC) and phenol, pH-value, particle size, viscosity, relative density, assays of the iron, toltrazuril and phenol, free iron, unspecified and total degradation products, extractable volume, sterility and bacterial endotoxins.

In general, the analytical methods used have been sufficiently described and appropriately validated in accordance with the relevant VICH guidelines. Relevant details for the particle size measurement and the apparatus used are provided. The HPLC method for assay and impurities has been appropriately validated and all known impurities of toltrazuril are well separated. Furthermore, the toltrazuril degradation pathway is well known and the method is therefore considered as sufficiently stability indicating.

Satisfactory information regarding the reference standards used for the assay of toltrazuril and the determination of related impurities, as well as the assay of phenol, has been presented.

Batch analysis results are provided for three production batches. The results confirm the consistency of the manufacturing process and its ability to manufacture the finished product to the intended product specification.

Stability

Stability data on three production batches of finished product stored for 24 months at 5 °C, 25 °C/60% RH and 30 °C/75% RH, and for 6 months at 40 °C/75% RH were provided.

The humidity of the intermediate storage condition is higher than the VICH requirement, but acceptable as it is more challenging.

The batches of finished product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance and colour, extractable volume, redispersibility, pH value, particle size distribution, viscosity, relative density, free iron, any unspecified degradation product, total degradation products, assay (toltrazuril, iron, phenol), bacterial endotoxins and sterility. In addition the container-closure integrity was tested.

All vials tested in the stability study were stored horizontally in order to ensure sufficient contact of the formulation with the rubber stopper.

In-use stability studies were performed on three production batches. The stability of the product was investigated at 4, 7, 14, 21 and 28 days after opening the vial.

Samples were tested for appearance, redispersibility, pH value, particle size distribution, viscosity, free iron, any unspecified degradation product, total degradation products, assay (toltrazuril, iron, phenol) and preservative efficacy. Preservative efficacy was tested at days 7 and 28.

The two batches were also exposed to light as defined in the VICH guideline GL5 (on photostability testing of new veterinary drug substances and medicinal products).

In addition, one of the production batches was exposed to stress conditions. Samples were stored for 7 days at +60 °C or –20 °C. Furthermore, samples were stored for 7 days at –20 °C, one day at +5 °C and another two days +60 °C. The product was investigated after 2 and 7 days at 60 °C, 7 days at -20 °C, and after 10 days of freeze-thaw cycles.

Samples were tested for appearance, redispersibility, pH value, particle size distribution, viscosity, free iron, any unspecified degradation product, total degradation products, and assay (toltrazuril, iron, phenol).

During all the stability studies no significant changes have been observed.

Based on the available stability data, the proposed shelf life of 3 years and in-use shelf life of 28 days, without any special storage conditions, as stated in the SPC, are acceptable.

Overall conclusions on quality

The finished product is a fixed combination product presented as an aqueous suspension for injection containing 182 mg of iron (as gleptoferron) and 36.4 mg of toltrazuril per ml as the active substances.

Other ingredients are phenol, polysorbate 20 and polysorbate 80, sodium chloride and water for injections.

The product is presented in clear silicon-coated glass Type II vials with chlorobutyl rubber stoppers and aluminium caps containing a nominal volume of 100 ml, as described in section 6.5 of the SPC. The materials comply with the relevant Ph. Eur. and/or EU requirements. The choice of the container-closure system has been validated by stability data and is adequate for the intended use of the product.

The product is manufactured aseptically. The development of the aseptic production process has been comprehensively described. Before aseptic processing, toltrazuril is sterilised by a validated gamma-irradiation process in compliance with the relevant guidelines.

The manufacturing process is considered as non-standard. The choice of a non-terminal sterilisation process is justified according to the Annex to the Note for guidance: Development pharmaceutics for veterinary medicinal products: Decision trees for the selection of sterilisation methods

(EMEA/CVMP/065/99) and is acceptable. Four validation batches have been produced and evaluated according to the validation plan. The manufacturing process and in-process controls have been described in detail, including maximum holding times and mixing times/speeds.

Gleptoferron is solely manufactured as an intermediate product (gleptoferron 20% Fe = GleptoMed 20% Fe) that contains the excipients water and phenol in addition. There is no clear distinction between the manufacture of the active substance and that of the intermediate product. Complete scientific documentation related to the manufacture and the quality assurance of the intermediate has been provided.

Full information on the active substance toltrazuril is provided in the dossier. There are two active substance manufacturers. Sufficient information on the manufacture of the active substance has been provided. The specifications for the starting materials, raw materials, solvent and reagents are appropriate.

Batch analyses results of batches from both production sites of the toltrazuril demonstrate compliance with the proposed active substance specification.

The toltrazuril stability results indicate that it is very stable (even after gamma-irradiation sterilisation) and the proposed retest period is justified.

The excipients are well known pharmaceutical ingredients and their quality is compliant with their respective current Ph. Eur. monographs. Sodium chloride, polysorbate 20 and polysorbate 80 are additionally tested for microbial purity.

Data have been presented to give reassurance on TSE safety.

The finished product specifications proposed for use at release and at the end of shelf life are generally acceptable. The finished product specifications include parameters relevant to the dosage form.

Batch analyses results of three production batches demonstrate compliance with the proposed finished product specification.

Based on the available stability data, the proposed shelf life of 3 years and an in-use shelf life of 28 days, without any special storage conditions, as stated in the SPC, are acceptable.

Sufficient and clear information has been provided in the dossier to support the authorisation of this medicinal product, and current regulations and guidelines have been taken into account.

Part 3 – Safety

Baycox Iron contains a fixed combination of the antiprotozoal substance toltrazuril and the anti-anaemic substance, iron (as gleptoferron), which was not authorised for a veterinary medicinal product in the EU at time of submission of the application. A full safety file in accordance with Article 13b of Directive 2001/82/EC - a fixed combination application, has been provided.

Safety documentation

A comprehensive data package with (recently performed) studies has been presented by the applicant. The pharmacological and toxicological properties of toltrazuril and iron have been characterized and described in the dossier based on available literature and laboratory studies performed by the applicant. Parts of these studies have already been evaluated by CVMP in the context of establishing MRLs for toltrazuril.

Pharmacodynamics

See part 4.

Pharmacokinetics

See part 4.

Toxicological studies

Single dose toxicity

In the context of the establishment of MRLs, CVMP concluded that the acute oral toxicity of toltrazuril was low: LD_{50} close to 2000 mg/kg bw in rats and higher than 5000 mg/kg bw in mice. For this procedure, acute dermal, inhalation and intravenous studies were presented on the active substance toltrazuril. No adverse effects were observed upon acute dermal and inhalation exposure; a dermal LD_{50} of higher than 5000 mg/kg bw was observed in rats. Acute intravenous injection resulted in moderate toxicity; intravenous LD_{50} values of about 100 mg/kg bw were observed in mice.

An acute oral study on the main metabolite toltrazuril sulfone was presented. The acute oral toxicity of toltrazuril sulfone was low.

In addition, acute oral and dermal studies with the fixed combination were provided. No mortality was observed following oral and dermal exposure up to the maximal dose of the product of 2000 mg/kg bw. Except for loss in body weight of some animals during the first week after dermal exposure, no other adverse effects were noticed.

Acute toxicity studies on iron dextran gleptoferron were not presented. It is noted that anaphylactic reactions may occur upon exposure. Acute effects after injection will be mainly limited to local reactions and hypersensitivity reactions, the latter however can be very severe.

Repeat dose toxicity

Repeat dose toxicity studies were performed with toltrazuril for up to 3 months in rats and dogs and for up to 4 weeks in mice. Repeated dose toxicity studies on toltrazuril via the dermal or inhalation route were not provided.

The most relevant studies are summarized below. The other studies displayed similar adverse effects, mostly reduced bodyweight, effects on liver (enzyme activity, organ weight, glycogen stores, plasma cholesterol) and haematological changes (reduced erythrocytes, haemoglobin and haematocrit levels).

In a 3-month oral toxicity study conducted in rats, toltrazuril was administered at dose levels of 0, 15, 60 and 240 ppm feed (approximately 0, 1.1, 4.2 and 16.6 mg/kg bw/day in males and 0, 1.2, 4.7 and 17.4 mg/kg bw/day in females). At the highest dose, the weight gain and the daily food intake were significantly decreased. Slight effects on haematological parameters and disturbances of liver function were also observed. At 15 mg/kg feed variations in some parameters, although statistically significant, were not considered relevant because they were not dose-related. The dose of 15 mg/kg feed (1 mg/kg bw/day) can be retained as the NOEL for this study, as concluded by CVMP in the context of the establishment of MRLs.

A 3-month oral toxicity study was conducted in dogs treated with toltrazuril at doses of 0, 1.5, 4.5 and 13.5 mg/kg bw/day. The highest dosage induced a significant increase in the weight of the heart without any histological changes or circulatory disturbance. The mean weight of the testes and the weight of the prostate were decreased. The NOEL retained was 1.5 mg/kg bw/day, as concluded by the

CVMP in the context of the establishment of the MRLs.

Repeated dose toxicity studies were performed with toltrazuril sulfone administered for a period of 13 weeks via the diet of rats and dogs. In the context of the establishment of the MRLs, the CVMP concluded the following: the assessment of the toxicity of toltrazuril sulfone showed that this main metabolite is less toxic than the parent compound. This is reflected by the higher NOEL obtained in 3-month toxicity studies (11.2 mg/kg bw/day versus 1.1 mg/kg bw/day with toltrazuril in rats and 8.3 mg/kg bw/day versus 1.5 mg/kg bw/day with toltrazuril in dogs) and in a teratogenic study in rats (90 mg/kg bw/day versus 0.5 mg/kg bw/day with toltrazuril).

Studies on repeated dose toxicity have not been provided for iron dextran gleptoferron. In the Martindale the following information can be found: unwarranted parenteral iron therapy will result in iron overload and excess storage of iron (haemochromatosis) in the long term. The consequences of this include liver and endocrine dysfunction and heart disease. Regarding the pharmacology and toxicology of iron, the safety profile is well established. Based on the low toxic potential, no MRLs were required. Therefore, no further data on pharmacology and toxicology are required to decide on user and consumer safety. The information submitted by the applicant may be considered appropriate.

There are no repeated dose toxicity studies with the fixed combination. However, in line with the CVMP guideline on fixed combination products (EMEA/CVMP/83804/2005), this was not considered necessary because interactions between the active substances and/or excipients or the possibility of masking toxicity are not expected to occur.

Tolerance in the target species of animal

See part 4.

Reproductive toxicity

Study of the effect on reproduction

A 2-generation study in rats receiving toltrazuril via the diet at doses of approximately of 0, 0.3, 1.25 and 5 mg/kg bw/day showed that the number of still-born pups was increased in all treated groups, but a statistical significance was only observed at the highest dosage and for the first generation of the lowest dose group. Due to these equivocal results, 0.3 mg/kg bw/day was retained as LOEL, as concluded by the CVMP in the context of the establishment of the MRLs.

There are no reproductive toxicity studies on toltrazuril sulfone or iron dextran gleptoferron.

Study of developmental toxicity

In the context of the establishment of the MRLs, CVMP reported the following on the developmental toxicity of toltrazuril:

Wistar strain rats received toltrazuril at doses of 0, 3, 10, 30 mg/kg bw/day (tylose suspension) and 0 and 1 mg/kg bw/day (in a supplemental study). Teratogenicity and embryotoxicity such as dysplasia of long bones, hydrops, cleft palate, microphthalmia were observed at the highest dose. When compared to the controls, a statistical decrease in the number of live foetuses was reported at 3 and 10 mg/kg bw/day. As a slight increase in the resorption was seen at 1 mg/kg bw/day, a LOEL of 1 mg/kg bw/day was retained for this study.

Sprague-Dawley rats were given 0, 1, 3, 10 and 30 mg/kg bw/day of toltrazuril (in carboxymethylcellulose). In this study, NOELs of 3 mg/kg bw/day and 10 mg/kg bw/day were retained for maternotoxicity and embryotoxicity.

Rabbits received toltrazuril at doses of 0, 1, 3 and 10 mg/kg bw/day. A significant increase in the number of abortions was reported at 3 and 10 mg/kg bw/day. As three litters showed runts at the lowest level, no NOEL was retained.

In a second rabbit study, doses of 0, 0.5, 0.75, 1 and 2 mg/kg bw/day of toltrazuril were given to rabbits. At 2 mg/kg bw/day, a significantly increased number of fused sternebrae was reported. As a significant increase of placental weights was reported at 0.75 mg/kg bw/day and 1 mg/kg bw/day, the lowest dose of 0.5 mg/kg bw/day was retained as the NOEL.

In a developmental toxicity study (GLP), toltrazuril sulfone was administered to rats by oral gavage at dose levels of 0, 10, 30, 90 and 300 mg/kg bw/day during GD6-15. Reduced food consumption was observed during the full treatment period, and reduced bodyweight was observed during GD11-15 in high dose females. No treatment-related effects on reproduction parameters were noticed. No treatment related external, visceral, skeletal abnormalities were observed. In the high dose groups, slightly delayed ossification was noticed. NOAELs for maternal toxicity and embryo foetal toxicity of 90 mg/kg bw/day could be derived from this study.

There are no developmental toxicity studies on iron dextran gleptoferron. However, the CHMP considered that, 'during pregnancy, allergic reactions are of particular concern as they can put both the mother and unborn child at risk. Intravenous iron medicines should therefore not be used during pregnancy unless clearly necessary. Treatment should be confined to the second or third trimester, provided the benefits of treatment clearly outweigh the risks to the unborn baby' (EMA/377372/2013).

Genotoxicity

In the context of the establishment of the MRLs, the CVMP reported the following on the mutagenicity of toltrazuril:

Toltrazuril gave negative results in five *in vitro* tests (Ames tests, mammalian cell mutation in Chinese hamster ovary (CHO) cells at the HPGRT locus, chromosomal aberrations test, Chinese hamster Ovary (CHO) cells and in unscheduled DNA synthesis in rat primary hepatocytes) and in one *in vivo* test (micronucleus test after oral administration).

A study on DNA-adduct formation in rat uterus after single oral doses of 300 or 600 mg/kg bw or repeated administration of 30 mg/kg bw/day for 7 days gave negative results. As the test used was not validated and due to deficiencies of the study, these results should be taken with caution. However, the CVMP overall concluded that toltrazuril was not mutagenic.

The metabolite toltrazuril sulfone was tested *in vitro* and *in vivo* for its potential genotoxic activity. The *Salmonella* tests in bacteria did not show any evidence for mutagenicity. Testing of toltrazuril sulfone in (GLP-compliant) HPGRT tests in Chinese hamster ovary cells (+/- S9) also did not show a positive response. An *in vitro* UDS-assay with primary rat hepatocytes gave negative results. No

indication for a clastogenic effect was found in an *in vivo* mouse micronucleus test (GLP-compliant). It can be concluded that toltrazuril sulfone is not genotoxic.

Iron dextran has been previously evaluated by EFSA for its safety and efficacy as a feed additive in piglets: "Genotoxicity of several iron compounds, including iron dextran, was evaluated in *Salmonella Typhimurium* (TA97a, TA98, TA 100, TA102 and TA1535) at concentrations up to 10 mg of compound, with and without metabolic activation, both by the incorporation and pre-incubation methods (Dunkel et al., 1999). Iron dextran did not show mutagenic effects. In L5178Y mouse lymphoma cells, iron dextran did not show mutagenic activity at concentrations up to 175 µg/ml without metabolic activation; however, with metabolic activation there was a concentration-dependent increase in the number of mutants at the two highest tested concentrations (8.75 and 17.5 µg/ml) (Dunkel et al.,

1999). This is a common feature of some transition metals, as iron, and can be expected to occur *in vivo* only when high concentrations of free ions are present. In normal conditions, iron is bound to proteins such as transferrin, ferritin and haemosiderin." When considering the consumer, no significant levels of free iron ions are expected.

Carcinogenicity

In a 2-year carcinogenicity study in mice (GLP), mice were treated with toltrazuril at doses (via the diet) of 0, 9.9, 41.4 or 95.2 mg/kg bw/day for males and 0, 11.9, 47.2 or 106.1 mg/kg bw/day for females. No treatment-related effects were observed with respect to neoplastic lesions.

In the context of establishment of the MRLs, the CVMP reported the following: Toltrazuril was tested in rats with dose levels of approximately 0, 1, 3, 10 mg/kg bw/day. In female rats, toltrazuril increased the incidence of endometrial adenocarcinomas significantly at the highest dosage (23/50 at 10 mg/kg bw/day; 6/50, 4/50 and 8/50 at 0, 1 and 3 mg/kg bw/day). At the highest dose, a reduction in the number of mammary tumours and hyperplasia in the pituitary was noticed in these rats. However, considering the significant increase in total incidence of pre-neoplastic and neoplastic lesions of the uterus for the two highest dosages (45 and 37 at 10 and 3 mg/kg bw/day versus 14 and 13 in the lowest and control groups), 3 mg/kg bw/day was retained as the threshold level for neoplastic tumours, while 1 mg/kg bw/day was retained as threshold dose for tumour promotion, i.e. pre-neoplastic lesions. Imbalance in the female hormone system was suggested to be involved in this tumourigenic mechanism.

Studies on the carcinogenicity of toltrazuril sulfone were not provided.

Studies on the carcinogenicity of iron dextran gleptoferron were not provided. Iron dextran has been previously evaluated by EFSA for its safety and efficacy as a feed additive in piglets and stated the following: "The National Toxicology Program (NTP) has assessed iron dextran complex in its use as injectable human medicine (usual daily dose is 1–5 ml (50–250 mg of iron)). The data available from epidemiological studies were inadequate to evaluate the relationship between human cancer and exposure specifically to iron dextran complex. However, the NTP concluded that iron dextran is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals which developed sarcoma at the injection site after repeated administrations (NTP, 2014). This finding is consistent with the effect of high local concentration of iron ions at the site of injection, which is not achieved with the oral administration of iron dextran." IARC has classified iron dextran as category 2B, possibly carcinogenic to humans http://monographs.iarc.fr/ENG/Classification/ClassificationsAlphaOrder.pdf.

Studies of other effects

Skin irritation and skin sensitisation studies with toltrazuril were negative. In one out of two eye irritation studies, toltrazuril showed slight eye irritation up to 72h, however this was fully reversed at day 7.

Skin and eye irritation and skin sensitisation studies with the fixed combination product were negative.

There are no skin and eye irritation and skin sensitisation studies on iron dextran gleptoferron. With respect to iron dextran, local reactions and anaphylactic reactions have been described.

Toltrazuril has been developed exclusively for veterinary use. Therefore, no human data are available.

Iron is used in human medicine for the treatment of iron deficiency. The formulation of iron as iron dextran is mainly used as injectable formulations. The most serious effects following the use of parenteral iron are hypersensitivity reactions which can be fatal. This led the European Medicines

Agency to review all the safety data of iron-containing products and to propose additional warnings in product literature.

Excipients

Phenol, polysorbate 20 and polysorbate 80 are common excipients in injectable formulations. All these excipients are considered as of minor importance compared to the active ingredients toltrazuril and iron. For phenol, a TDI (tolerable dietary intake) of 0.5 mg/kg bw/day (EFSA) is available. It is noticed that the critical effect for this TDI is a reduced maternal body weight gain with a BMDL10 of 52 mg/kg bw/day (developmental toxicity study, rat). Based on this, it can be considered that the general toxicity is mainly determined by toltrazuril. However, EFSA also derived for phenol a BMDL05 of 98.7 mg/kg bw/day for developmental toxicity (increased pup death in the second generation; rat, two-generation study).

User safety

A user safety risk assessment has been conducted in accordance with CVMP guideline EMEA/CVMP/543/03-Rev.1.

Toxicological reference values:

The following findings should in principle be taken into account in the (quantitative) risk characterisation when considering active substance toltrazuril:

For acute and short-term exposure: The NOEL of 1 mg/kg bw/day (3-month oral rat) is considered as the most appropriate toxicological reference value for acute and short-term oral toxicity.

It is noted that no dermal repeated dose studies with toltrazuril or the product are available, only acute dermal toxicity studies with toltrazuril or the product are available. Although no adverse effects were observed in these acute dermal toxicity studies, these studies (focussing on mortality) should not primarily be used for the quantitative risk characterisation as these are limited in the parameters examined. In the absence of dermal repeated dose studies, the CVMP considers the oral NOEL of 1 mg/kg bw/day (3-month rat; effect on liver function and haematological parameters) as the most appropriate toxicological reference value. There are no data on dermal bioavailability in order to convert the NOEL; therefore the worst case, that is, the oral NOEL, is used as a dermal NOEL, assuming that dermal bioavailability will be similar, or at least not be higher than, oral bioavailability.

Toxicological reference values for long-term exposure assessment are not necessary, as this product is administered by professionals who are only expected to be accidentally exposed on single occasion(s).

For assessment of developmental toxicity: The occurrence of developmental effects is not restricted to repeated exposure only, as it may occur during the critical window of development of a foetus. The LOEL of 0.3 mg/kg bw/day (rat, 2-generation study, stillborn pups) is considered as the most appropriate toxicological reference value. This toxicological reference value can be used for oral as well as dermal exposure for a similar reason to the justification described above.

Local effects:

Skin irritation and skin sensitisation studies with toltrazuril were negative. Skin and eye irritation and skin sensitisation studies with the fixed combination product were negative.

There are no toxicity studies on iron dextran gleptoferron. Acute effects after injection are expected to be limited to local reactions and anaphylactic reactions based on information on iron dextran. Also, iron dextran is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals, which developed sarcoma at the injection site

after repeated administrations (NTP).

For phenol, a $BMDL_{05}$ of 98.7 mg/kg bw/day for developmental toxicity (increased pup death in the second generation; rat, two-generation study) is available.

Exposure:

The users of this product are professionals (including veterinarians and farmers and/or their staff). As this product is used in a professional setting, accidental exposure by children is considered to be negligible.

The tasks and situations that lead to exposure have been well characterized and include filling the syringe, administering the product by injection and disposal of the syringe.

The most relevant routes of exposure are dermal exposure and accidental self-injection. During normal administration of the product, when elementary personal hygiene is maintained by a professional, oral exposure (via hand-to-mouth contact) is considered negligible. This is also considered for eye exposure via hand-to-eye contact. However, eye exposure via accidental release of the product from a charged syringe may be possible. Exposure via inhalation is considered unlikely.

Dermal exposure: For dermal exposure, it can be assumed that a professional user will spill accidentally only a small volume; one drop (i.e. 50 µl) is a reasonable worst case. However, several piglets are treated at the same time. As a reasonable worst case 3 droplets, i.e. 150 µl, are considered. This would result in a dermal exposure of 0.09 mg/kg bw for toltrazuril and 0.01 mg/kg bw for phenol.

Accidental self-injection: Accidental self-injection is considered to occur incidentally. A volume of 10%, or maximally 0.5 ml, is considered to be a reasonable worst case. This would result in an exposure of 0.3 mg/kg bw for toltrazuril and 0.04 mg/kg for phenol.

Qualitative risk characterization:

Based on the results of the skin irritation and skin sensitisation studies with toltrazuril or the final formulation, no irritating effects are anticipated for this product.

With respect to iron dextran, local reactions and anaphylactic reactions have been described. Iron dextran might cause sarcomas as these were observed in experimental animals which developed sarcomas at the injection site after repeated administrations. As repeated injections are not anticipated for a professional user, this is not considered a reasonable risk when this product is used in accordance with the product information.

Quantitative risk characterization:

Dermal exposure

The margin of exposure (MOE) calculated for toltrazuril due to spilling was 11. This is below the acceptable value of 100. However, it was noted that the NOEL used to calculate the MOE is an oral NOEL derived from a 3-month repeated dose study (slight effects on the haematological parameters and disturbances of the liver function); and it was assumed that the dermal NOEL is similar to the oral NOEL, however, data from an in vitro study indicate that dermal absorption of toltrazuril may be limited (0.34% for human skin); moreover, the user is a professional for whom it is assumed that personal hygiene is maintained, such as washing hands. Therefore, the risks for systemic toxicity concerning dermal exposure are acceptable.

When considering developmental toxicity, the calculated MOE would be 3. This appears not to be acceptable, especially when considering that a LOAEL is used for this risk characterization. However, dermal absorption is expected to be low, resulting in an overestimate of the internal dermal exposure. It is therefore concluded that the risk for the unborn child with respect to dermal exposure will be

sufficiently mitigated if hands are washed.

Accidental self-injection

The MOE calculated for toltrazuril upon self-injection was 3. Although, it is noticed that the NOEL of 1 mg/kg bw is derived from a 3 month oral repeated dose study, the MOE is far below the acceptable value of 100. It is also noted that in a 4 day (dose-range finding; via diet) oral repeated dose study, lethargy, reduced food consumption, reduced body weight and liver effects were observed. Lethargy and apathy were observed from the second day of the study onwards. The NOEL derived for this study was 9.8/7.3 mg/kg bw (males/females). Considering the multiple use of this product, accidental self-injection and the occurrence of adverse acute effects is not negligible. Therefore, warning sentences should be in place.

When considering developmental toxicity, the calculated MOE would be 1. This is far below acceptable, especially when considering that a LOAEL is used for this risk characterization. Because of the multiple use of this product accidental self-injection is not negligible and may occur in the critical period of development of the foetus. Therefore, warning sentences considering developmental toxicity should be in place. With respect to phenol, no risk for developmental toxicity is anticipated.

Severe hypersensitivity reactions to intravenous iron products used during pregnancy can put both the mother and unborn child at risk. Therefore, a warning for pregnant women should be in place.

Risk communication:

Based on the assessment, the following warnings and safety measures are considered appropriate and are included in the SPC (and package leaflet):

- This product contains iron (as gleptoferron complex), which has been associated with anaphylactic reactions after injection. People with known hypersensitivity to iron (as gleptoferron complex) should avoid contact with the veterinary medicinal product.
- Accidental self-injection may cause adverse effects. Care should be taken to avoid accidental self-injection. In case of accidental self-injection, seek medical advice immediately and show the package leaflet or the label to the physician.
- This product may be harmful for the unborn child. Pregnant women and women intending to conceive should avoid contact with the veterinary medicinal product, especially accidental self-injection.
- Wash hands after use and/or spillage.

Based on the above risk assessment, the CVMP concluded that the product does not pose an unacceptable risk to the user when used in accordance with the SPC.

Environmental risk assessment

A Phase I environmental risk assessment (ERA) was provided according to the CVMP/VICH guidelines. The Predicted Environmental concentration in soil was calculated for iron and toltrazuril. These calculations are performed in accordance with VICH guideline GL6 and the CVMP guideline on the Environmental Impact Assessment for Veterinary Medicinal Products in support of the VICH guidelines GL6 and GL38 (EMEA/CVMP/ERA/418282/2005-Rev.1). Since for piglets of three days old no default value on body weight is provided in the guideline, these values were obtained from public literature. The calculations are based on the weight of a three days old piglet of 2.3 kg, 26.8 piglets per place per year and a nitrogen production of 26 kg per place per year for the existing target animal category 'Sow + litter', considering the piglets stay with their mother until weaning.

Exposure assessment	Value (µg/l – µg/kg)
PEC soil	11

Phase I:

The environmental risk assessment can stop in Phase I and no Phase II assessment is required because:

Iron is a natural substance, the use of which will not alter the concentration or distribution of the substance in the environment.

The initial predicted environmental concentration of toltrazuril in soil ($PEC_{soil, initial}$ = 11 µg/kg) is less than 100 µg/kg.

Conclusions on the environmental risk assessment

An ERA was provided according to the CVMP/VICH guidelines. Based on the data provided, the ERA can stop at Phase I, as none of the Phase I criteria are met. Baycox Iron is not expected to pose a risk for the environment when used according to the SPC.

Residues documentation

MRLs

The MRL status of the constituents of Baycox Iron is as follows:

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissues	Other provisions	Therapeutic classification
Toltrazuril	Toltrazuril sulfone	All mammalian food producing species	100 µg/kg 150 µg/kg 500 µg/kg 250 µg/kg	Muscle Fat Liver Kidney	For porcine species the fat MRL relates to 'skin and fat in natural proportions'. Not for use in animals from which milk is produced for human consumption. Not for use in animals from which eggs are produced for human consumption.	Antiparasitic agents/ Agents acting against protozoa
		Poultry	100 µg/kg 200 µg/kg 600 µg/kg 400 µg/kg	Muscle Skin and fat Liver Kidney		
Iron (as gleptoferron)	The "No MRL required" classification for iron dextran and iron glucoheptonate is considered to apply to gleptoferron as gleptoferron is expected to release iron dextran and iron glucoheptonate.					

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.

Residue studies

Pharmacokinetics

Several pharmacokinetic studies were available for the target species, pigs. The main findings were as follows: Oral absorption of toltrazuril is almost complete with oral bioavailability of approximately 69%. Toltrazuril was mainly metabolised into a sulfoxide and sulfone metabolite. Excretion was primarily via the faeces, but excretion via the urine was also observed. The uptake of iron from the diet is dependent on the iron requirement of the body, i.e. body iron status, iron content and composition of food. Iron is not readily eliminated; most of it is reused, while only small amounts are eliminated.

See Part 4 for pharmacokinetic information following parenteral administration.

Depletion of residues

One GLP compliant tissue depletion study in piglets was designed as a key residue study to determine the concentrations of the marker residue toltrazuril sulfone in injection site muscle (core+ring), loin muscle, liver, kidney and skin+fat of the target animal. In addition to the marker residue toltrazuril sulfone, the parent compound toltrazuril and the metabolite toltrazuril sulfoxide were also analysed. The target animals were treated at the dose and by the route of administration intended for marketing. A sufficient number of animals (4/group) and slaughter time points (4) were investigated. Piglets (3 days; 1.2-2.5 kg (m), 1.9-2.6 kg (f)) were treated with a single intramuscular injection of 20 mg toltrazuril/kg bw and 100 mg iron/kg bw. Tissue residues were determined at 28, 42, 56, 75 days post-administration using a validated LC-MS/MS method (LOQ: 10 µg/kg). The highest concentrations of the marker residue toltrazuril sulfone were observed 28 days after administration of the product, the first time point measured, with highest residue levels observed in liver, followed by kidney and skin+fat. Lower concentrations were found in muscle, including the injection site. At the next sampling point (day 42), residues in the liver and kidneys and were below their respective MRLs. Residues in muscle (injection site-core, injection site-ring, loin muscle) and skin+fat were below the MRL at day 56. No clear difference in residue levels between injection site muscle (core+ring) and loin muscle were noticed, which can be explained by the even distribution of the residues throughout all muscle tissues at the first day of slaughter, which is 28 days after administration. The study was considered suitable for the withdrawal period determination.

Withdrawal periods

Based on the data from the residue depletion study, including the correction of the residue levels of toltrazuril sulfone for procedural recovery, a withdrawal period of 53 days was calculated. The withdrawal determining tissue was the injection site (ring), however the withdrawal period resembles the withdrawal periods calculated for the injection site (core) of 49 days and muscle (51 days) and therefore appears to reflect the even distribution of the marker residue throughout the muscle tissues.

Overall conclusions on the safety and residues documentation

Toxicology: an extensive list of toxicity studies has been provided on the individual active substances in the product. This is in line with the CVMP guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005). The data presented are considered adequate to characterise the toxicity

profile of the active substances. Many of these studies have already been assessed by the CVMP in other procedures, such as the MRL procedures.

User safety: a user safety assessment in line with the relevant guidance document has been presented. The worst case scenario for user safety is accidental self-injection, especially for pregnant women. The proposed measures and safety advice/warnings are considered adequate to mitigate the risk to the user.

Environmental risk assessment: an appropriate environmental risk assessment was provided. Baycox Iron is not expected to pose a risk for the environment when used according to the SPC.

Residues: an adequate residue depletion study in piglets has been performed. Based on the marker residue data and the MRLs established by the CVMP, a withdrawal period for edible tissues of 53 days for piglets was calculated and justified.

Part 4 – Efficacy

Justification of the fixed combination

Baycox Iron is a fixed combination containing toltrazuril and iron (as gleptoferron) in a suspension for injection for piglets, containing 36.4 mg/ml toltrazuril and 182 mg/ml iron (as gleptoferron) and is intended to be administered in 3 day old piglets (i.e. 48 to 72 hours after birth). The dose to be administered is 20 mg toltrazuril/kg body weight and 100 mg iron (as gleptoferron complex)/kg body weight by intramuscular injection. This is equivalent to a dose volume of 0.55 ml/kg bw.

Baycox Iron is indicated for use in neonatal piglets for the concomitant prevention of clinical signs of coccidiosis (such as diarrhoea) on farms with a confirmed history of coccidiosis caused by *Cystoisospora suis* (Syn. *Isospora*), as well as prevention of iron deficiency anaemia. To obtain a maximum benefit, animals should be treated before the expected onset of clinical signs, i.e. in the pre-patent period.

Toltrazuril is already authorised as an oral suspension for preventive treatment of *C. suis* infections in piglets, and iron injections are authorised and routinely administered to piglets between the first and third day of life to prevent anaemia in most production systems throughout the world. A satisfactory justification for this fixed combination, in agreement with the CVMP Guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005), was provided. The following advantages justify the combination product versus the combined use of single substances:

- A clinical benefit, since almost 100% of fast-growing piglets need parenteral iron supplementation, and coccidiosis is a widespread disease. In farms where piglets are at risk of developing coccidiosis, these animals also need to be treated with iron in their first days of life;
- A practical benefit for the breeder, i.e. an injection of iron associated with an oral administration of toltrazuril will be replaced by a single injection of the new fixed combination product.
- The new formulation will also improve animal welfare since neonatal piglets are manipulated very often during the neonatal period (navel cord care, teeth clipping, tail docking, ear notching for identification, castration of males and cross-fostering). Thus, this new product reduces one management procedure of piglets.

Pharmacodynamics

The pharmacodynamics of the active substances in Baycox Iron, iron and toltrazuril (both well-established active ingredients), have been thoroughly reviewed based on the available literature provided by the applicant. Toltrazuril is already authorised for use as single substance as an oral

suspension at the dose of 20 mg toltrazuril/kg bw in piglets on day 3-5 of life. Likewise, various (intramuscular) injectable iron mono preparation are authorised to be used in piglets during the first three days of life, at doses between 100-200 mg/piglet.

Toltrazuril, a symmetrical triazinone, has been reported to be effective against coccidia. It is a broad-spectrum anticoccidial substance, chemically unrelated to all other known anticoccidials. It affects the mitochondria and endoplasmic reticulum of the parasites and impairs nuclear division into schizonts and microgametocytes. As a result, reproduction of the parasites is considerably reduced.

Iron is an essential micronutrient for the function of all body cells. It plays a critical role in cell-cycle regulation, electron transport in the respiratory chain, DNA synthesis and other metabolic reactions. It is crucial for the function of oxygen-binding molecules such as haemoglobin (Hb), myoglobin, and many iron-containing enzymes including the cytochromes in the mitochondria. It is a constituent of haemoglobin and myoglobin and has a key role in enzymes, such as cytochromes, catalases, and peroxidases. Piglets have a low iron deposit at birth, and under intensive farming conditions there is insufficient oral iron uptake via their diet; as they have a high growth rate, supplementary iron is therefore necessary in the first days of life.

Development of resistance

The applicant performed a bibliographical search where generally resistance to toltrazuril in pigs was not reported for *C. suis* in the field. However, one report was provided confirming lack of efficacy of toltrazuril treatment for *C. suis* in piglets on a farm in the Netherlands in 2014. Also, resistance to toltrazuril is well-known for poultry coccidiosis. Because of the long-term use of toltrazuril and the absence of alternative treatments, resistance to toltrazuril in pig farms could become a problem as in poultry farms.

Therefore, this new fixed combination product must only be used when the indication is well justified (iron supply and treatment against coccidiosis). Nevertheless, there is no indication in the scientific literature of a sudden increase in the spread and severity of toltrazuril resistance in the past two years. Moreover, preventive measures against anticoccidial resistance such as the need for hygienic measures have been appropriately addressed in section 4.4 of the SPC.

Pharmacokinetics

To determine the plasma pharmacokinetic profile of iron, toltrazuril and its major metabolite, toltrazuril-sulfone, which is also clinically active on all endogenous parasite stages, the applicant performed two GLP compliant, pivotal plasma pharmacokinetics studies. Data on intravenous administration of toltrazuril to piglets was provided from a previously (1999) performed GLP study.

Studies were performed in three day-old piglets and two day-old piglets, representative for the target population and conducted in line with the CVMP guideline on the conduct of pharmacokinetic studies in the target animal species (EMEA/CVMP/133/99-Final). Methodology used in these studies was suitable to carry out pharmacokinetic studies, and can be summarized as follows:

The study in three day-old piglets was performed in 13 healthy animals, that all received the test item at a single intramuscular target dose of 20.0 mg toltrazuril per kg bw and 100 mg iron per kg bw. Iron pharmacokinetics was not studied, and the pharmacokinetics of toltrazuril were only studied in the combination product.

The study in two day-old piglets was performed in 30 animals that were divided in three groups. Ten animals received the test item at a single intramuscular target dose of 20.0 mg toltrazuril per kg bw and 100 mg iron per kg bw, ten animals received the oral positive control product containing toltrazuril alone

at a mean dose rate of 20.0 mg toltrazuril per kg (though, for animal welfare reasons, this group also received iron supplementation). The third group was only treated with iron, to obtain information on iron pharmacokinetics.

Blood samples were frequently collected up to a sufficient long period (Day -1 up to D85/D70). In both studies, the number of samples (19-22 samples per animal) was sufficient to adequately describe the plasma concentration-time profile.

Animals were monitored daily for their general health status. Main pharmacokinetic parameters for iron, toltrazuril and its metabolites toltrazuril-sulfoxide and toltrazuril-sulfone were determined in plasma.

Toltrazuril:

Following intramuscular administration, metabolism to toltrazuril sulfoxide and toltrazuril sulfone, and elimination from plasma are comparable to after oral administration, suggesting that clinical efficacy is at least comparable to oral administration of toltrazuril.

In the study in three day-old piglets, the mean concentration of peak plasma toltrazuril was 4.17 mg/l; reached in 5 days. The mean toltrazuril-sulfone plasma peak concentration was 6.23 mg/l, reached 15 days after injection of the parent drug. In the study in two day-old piglets, the mean concentration of peak plasma toltrazuril was 6.43 mg/l; reached in 5 days. The mean toltrazuril-sulfone plasma peak concentration was 8.08 mg/l, reached 11 days after injection of the parent drug. Slight differences in bioavailability and half-life observed within the two presented studies were most likely caused by biological variability. The plasma pharmacokinetics of both studies indicated that toltrazuril is absorbed slower when administered IM than when toltrazuril is administered orally (absorption is delayed by maximal three days, based on results derived from the two day-old piglet study). Clinical efficacy was however not negatively impacted by this finding.

In both studies, following intramuscular administration of the therapeutic dose rate of 20 mg toltrazuril per kg, bioavailability was higher than after oral administration (138% and 165%). However, target animal safety (TAS) evaluations did not reveal any safety concern that could be caused by this increase in bioavailability.

In the study in three day-old piglets, total plasma exposure of toltrazuril and toltrazuril-sulfone amounted to 1046 mg*h/l and 3868 mg*h/l, respectively. Toltrazuril and toltrazuril-sulfone were eliminated from plasma with a half-life of about 4 days and 7 days, respectively. In the study in two day-old piglets, total plasma exposure of toltrazuril and toltrazuril-sulfone amounted to 1244 mg*h/l and 4095 mg*h/l, respectively. Toltrazuril and toltrazuril-sulfone were eliminated from plasma with a half-life of about 3 days and 5 days, respectively.

Toltrazuril and its metabolites are mainly eliminated in faeces via biliary excretion and to a small extent via urine.

The applicant presented data for the evaluation of dose proportionality of toltrazuril plasma exposure after intramuscular injection at 3 different dose rates (5, 10, and 20 mg/kg). Results showed that extent of plasma exposure (AUC) increased proportionally between 5 and 10 mg/kg but less than proportionally up to 20 mg/kg (under-proportional increase). The plasma exposure profiles of toltrazuril and metabolites were very comparable with regard to shape of profiles giving no hint of any saturation processes or metabolic changes with increasing dose rates.

Iron:

After intramuscular injection, the iron complex is absorbed mainly into the lymphatic tissue, where it is split to release iron (III)-ions. Plasma iron concentration peaks within the first day reaching concentrations of 548 mg/l at 6 hours after injection. Free iron (III)-ions are removed from plasma and

stored as ferritin, with a half-life of approximately 8 hours. From 72 h onwards, this is followed by very slowly decreasing plasma concentrations with a calculated mean half-life of 960 h. The primary routes of iron excretion are via faeces and urine.

The pharmacokinetic profiles of iron administered intramuscularly to young piglets, as a mono-substance or in a fixed combination with toltrazuril (Baycox Iron), were evaluated in the study in two day-old piglets. The plasma pharmacokinetics of iron in the fixed combination or in the single-substance displayed a very comparable pattern, and the CVMP agreed that there were no indications for unintended drug-drug-interactions for the iron component for the fixed combination with toltrazuril.

Interactions between the two active substances:

No classical drug-drug interaction study was performed to investigate the impact of iron contained in Baycox Iron on the plasma PK profile of toltrazuril. However, unintended drug-drug interactions with iron do not appear to be present, and it is considered that intramuscular toltrazuril metabolism and elimination from plasma are comparable to oral administration. The Guideline on pharmaceutical fixed combination products (EMEA/CVMP/83804/2005) states, that '*only if the pharmacological data have clearly demonstrated no interactions between the active substances, justification for the dose selection can be based on data for each individual active substance'*. A three way study would normally be necessary to establish the absence of interaction between toltrazuril and iron, with one group receiving the fixed combination product and two groups receiving one of the active ingredients. However, introducing a group of piglets without iron administration is not possible considering that piglets, in all cases, need to receive this injection in the first days of life to avoid iron deficiency anaemia. As a conclusion, the absence of a group treated with toltrazuril alone is scientifically sound and is accepted.

In conclusion, the information provided by the applicant on the pharmacokinetics of this veterinary medicinal product is in line with the requirements established in the European legislation. Sufficient pharmacokinetics data is provided to conclude on the absence of unintended interactions between the two active ingredients of this fixed combination product.

Also, the kinetic profiles of each of the substances in the combination as well as kinetic profiles of the mono-substances have been provided. This allowed for accurate characterisation of the pharmacokinetics of the two active ingredients of this fixed combination product, toltrazuril and iron, in plasma.

Dose justification

Toltrazuril:

To prevent the clinical signs of coccidiosis in neonatal piglets, an oral dose of toltrazuril at 20 mg/kg bw is well-established, and the applicant proposed the same dose for the intramuscular application, based on the pharmacological profile of toltrazuril. An efficacious toltrazuril dose had not been established when the product is administered intramuscularly. However, the absence of unintended interactions between the active substances was sufficiently demonstrated in the pharmacokinetics studies provided. Also, the kinetic profiles of toltrazuril in the combination as well as kinetic profiles of the mono-product were demonstrated. In addition, the applicant referred to six clinical studies, including three field studies, which investigated the efficacy of different doses of the injectable formulation of toltrazuril and iron against coccidiosis caused by *C. suis*. Lower intramuscular doses between 1 and 15 mg/kg bw were explored with either dose determination studies or field studies. Higher doses than 20 mg/kg bw were not explored, which is acceptable considering efficacy was already observed at lower doses than 20 mg/kg bw, and considering the kinetic profile of toltrazuril after intramuscular injection.

When tested under controlled conditions in laboratory trials, intramuscular treatment with a low dose of toltrazuril (10 mg/kg bw) was shown to be effective: In one study, a maximum toltrazuril dose of 10 mg/kg bw prevented diarrhoea and quantifiable oocyst excretion. In a second study, all groups treated with toltrazuril (5-20 mg/kg bw) showed significantly less diarrhoea than the untreated control group ($p<0.0001$), with no quantifiable oocyst counts in all of the toltrazuril-treated groups. In another dose determination study, limited diarrhoea was observed in all three groups. However, the number of days with faecal score (FS) above 2 was significantly less in the two treated groups (5 or 10 mg/kg bw) compared to the untreated control group, and this study also did not observe quantifiable counts for oocyst excretion in both of treated groups.

However, a toltrazuril dose of 10 mg/kg bw proved insufficient in the field studies. Results of the field study that compared intramuscular doses of 20 mg/kg bw toltrazuril against 10 mg/kg bw toltrazuril, favoured the 20 mg/kg dose over the 10 mg/kg dose in terms of oocyst excretion, faecal score, and weight gain. Safety and efficacy of the 20 mg/kg dose was confirmed in the clinical field study.

The CVMP therefore accepted the proposed intramuscular dose of 20 mg/kg bw toltrazuril.

Iron:
Parenteral iron supplementation by intramuscular administration of 100 to 200 mg of iron dextran per piglet is a well-established practice in piglets between the first and third day of life. The proposed dose of iron (100 mg/kg bw) is based on the existing iron products approved for piglets.

The CVMP acknowledges the well-established veterinary use of gleptoferron in prevention of iron deficiency anaemia in neonatal piglets. The new product is intended to be administered in the same target population by the same route and at the same effective dosage as for the control product. In addition, studies provided by the applicant demonstrated no unintended interaction between the active substances in this product.

Overall, results of the included studies sufficiently justify the proposed intramuscular dose of toltrazuril at 20 mg/kg bw and iron at 100 mg/kg bw.

Dose determination / finding studies

The applicant presented three GCP-compliant dose finding studies. These studies were conducted using similar methods. All studies were controlled (negative control for toltrazuril), blinded, and performed in a randomised complete block design. In addition to these three laboratory studies, the applicant provided two field studies that also tested different doses of toltrazuril (5 and 10 mg/kg bw, and 10 and 20 mg/kg bw), one considered by the applicant as a 'dose confirmation study' and the other an additional, supportive controlled field efficacy study .

For the three laboratory studies, all piglets were artificially infected with *C. suis* on day two of life. Infection was performed by oral administration of approximately 1000 sporulated oocysts of a recent (2005) Austrian field isolate of *C. suis*. Infection was followed by intramuscular treatment with a sufficiently near-to-final formulation on day three of life. In all studies, the suspension contained different toltrazuril concentrations, and toltrazuril doses of up to 20 mg/kg bw were evaluated. In none of the studies the iron dose was according to the recommended dose. In two of these laboratory studies a slightly lower iron dose than proposed was used (91 mg iron/kg bw). This was due to product development. Since the difference with the proposed dose is low, this difference in iron dose is not expected to result in a clinically relevant outcome. In all cases, the control-groups received only an intramuscular iron injection at a dose of 100 mg/animal.

For all studies, primary efficacy criterion was quantifiable oocyst excretion; i.e. the "number of days with McMaster countable oocyst excretion (OPG) >0"; and the co-primary efficacy criterion was Faecal

consistency (Faecal Score (FS) of 1 to 4; (diarrhoea: FS>2)). Secondary variables were various safety parameters (adverse events, serious adverse events, mortality), clinical observations, weight gain and the presence of oocysts (autofluorescence-detectable oocyst excretion). For one of the laboratory studies ; haematological examination and faecal scores were also considered secondary variables.

The objective of the first Dose Determination Laboratory Study was to determine the effective dose and safety of the fixed combination against experimental infection with *Cystoisospora suis* in neonatal piglets. The study included 35 piglets that were divided into three dose groups. On D3, toltrazuril and iron were administered as a fixed combination at either 0, 5, or 10 mg/kg bw toltrazuril combined with 91 mg iron per kilogram bodyweight, respectively.

Quantifiable counts for oocyst excretion were not observed in any of the treated groups. The number of days (NOD) of oocyst shedding was 17.7% in the untreated control group. In all groups, some diarrhoea was observed, though the number of days with faecal scores (FS) >2 was significantly less in the two treated groups. The % of piglets with FS above 2 (at least once) was 58.3 (5 mg/kg bw) and 36.4 (10 mg/kg bw), respectively, compared to 100% in the untreated control group. Mean days with FS>2 in treated animals was 1.3 (5 mg/kg bw) and 0.6 (10 mg/kg bw) compared to 4 days in the control group. This study demonstrated that an intramuscular dose of 10 mg/kg bw toltrazuril was superior over a 5 mg/kg bw toltrazuril dose.

The objective of the second Dose Determination Laboratory study was to determine the (lowest) effective dose and to evaluate safety of the fixed combination against an experimental infection with *C. suis* in piglets, compared to a control group treated with commercial injectable iron only. Fifty-one piglets were divided in five groups (n=10 per group). On D3, four groups of animals received the fixed combination containing iron (91 mg/kg bw) and either 1, 2.5, 5 or 10 mg/kg bw toltrazuril, respectively; the fifth group only received iron (negative control).

The number of days of oocyst shedding in the toltrazuril treatment groups was 0 in the 5-10 mg kg bw toltrazuril groups (compared to approximately 42 and 11 days at lower doses, and 21 in the control group). The % of piglets with faecal scores (FS) above 2 (at least once) was 0 in the 10 mg/kg bw toltrazuril group (compared to 100% in the untreated control group). The mean number of days piglets had a FS >2 was 0 only in the group treated with 10 mg/kg bw toltrazuril. The results indicate that 10 mg/kg bw toltrazuril is superior to 5 mg/kg bw in an experimental infection with *C. suis*.

The objective of the third GCP compliant efficacy study , was to compare the efficacy of different dose rates of a fixed combination toltrazuril and iron on clinical, haematological, parasitological and safety parameters in suckling piglets experimentally infected with *Isospora suis*.

Fifty animals were divided into four groups (n=12). At D3, the IVP was administered at 5, 15 or 20 mg/kg bw (groups B, C, and D, respectively). A fourth group (A) was only treated with iron (no toltrazuril) at a dose of 100 mg/kg bw (negative control). Since in this case a fixed combination of toltrazuril and iron was used, different dosages were only achieved by increasing the injection volume, thus resulting in a different dosage of both toltrazuril as well as iron: Group B received 33 mg/kg bw iron and 5 mg/kg bw of toltrazuril, Group C received 100 mg/kg bw iron and 15 mg/kg bw toltrazuril, and Group D received 133 mg/kg bw iron and 20 mg/kg bw toltrazuril. Thus, in this study, the three groups all received different dosages of iron (none dosed according to label). However, the main aim of this study was to determine an adequate dose of toltrazuril in the combination product, and the efficacy of toltrazuril was not affected by the difference in iron dose.

Concerning the primary efficacy parameters, all groups treated with the fixed combination performed significantly better than the negative control group (iron-only) ($p<0.0001$) without significant differences among the different doses of the IVP. All toltrazuril treated groups showed significantly less diarrhoea than the control group ($p<0.0001$) and the animals treated with 15 and 20 mg/kg bw

toltrazuril, showed significantly less diarrhoea than the animals treated with 5 mg/kg bw toltrazuril. Mean days FS>2 was 0.1 in the group treated with 20 mg/kg toltrazuril. In the toltrazuril-treated groups, no quantifiable oocyst counts were observed.

The dose determination study also assessed the efficacy of the product against iron deficiency anaemia at doses of 100 (A), 33 (B), 100 (C) and 133 mg iron/kg bw (D). Plasma iron was measured at D1, D8 and D 22. At all three measuring points, plasma iron showed high variability between and within groups. For the most relevant haematological parameters (RBC, HB, Ht), no significant differences were observed. Dose dependant plasma-iron levels were detected:

- At study day 1, mean plasma iron in the control group A was 76.3 (SD 18.1) compared to Group B: 77.2 (SD: 25.8), Group C: 93.4 (SD38.3), Group D: 85.6 (SD 31.2).
- At study day 8, mean plasma iron in the control group was 180.1(SD 56.7) compared to Group B: 49 (SD: 30.9), Group C: 199 (SD 29.4), Group D: 236 (SD 66.8).
- At study day 22, mean plasma iron in the control group was 70.4 (SD 40.4) compared to Group B: 14.9 (SD: 4.1), Group C: 30.9 (SD8.9), Group D: 56.9 (SD 30.3).

It can be concluded that for all treatment groups, the fixed combination proved efficacious against induced infections with *Cystoisospora suis*. Although dose dependant plasma-iron levels were detected between the groups, these were not reflected by significant differences for the relevant haematological parameters between study days 8 and 22.

Dose confirmation studies

The applicant provided two field dose confirmation studies to confirm the proposed dose of toltrazuril and iron, one negatively and one positively controlled.

The objective of the blinded, negatively controlled multicentre field dose confirmation study, was to confirm the effective dose and safety of the fixed combination against natural infection with *Isospora suis* in neonatal piglets in farms with a history of coccidial infection. This study also evaluated efficacy of the product against iron deficiency anaemia, by evaluating HB, HT, and RBC on D3, D13 and D21.

Piglets (n=506) with normal health status were included in this study and divided into three groups that received either commercial iron injection only; 5 mg/kg bw toltrazuril and 91 mg/kg bw iron; or 10 mg/kg BW toltrazuril and 91 mg/kg bw iron. This study did not use the final formulation, but a sufficient (bioequivalent) close-to-final formulation was used.

Results of this study demonstrated that all treatment groups were significantly superior over the control group concerning primary variables ("number of days with McMaster countable oocyst excretion >0" and faecal consistency as a co-primary variable). The group treated with 10 mg/kg was only significantly superior over the group treated with 5 mg/kg toltrazuril for the criteria "number of days with McMaster countable oocyst excretion", but not for faecal consistency. No statistically significant differences in laboratory values (hemoglobin, haematocrit and red blood cells) were observed between the different groups of animals.

From this study, it can be agreed that the fixed combination appears efficacious in preventing coccidiosis and balancing iron requirements at both of these doses, but that the higher dose of 10 mg toltrazuril/kg bw appears to be more effective in coccidiosis prevention, since oocyst shedding was significantly less with 10 mg/kg bw toltrazuril (mean days oocyst >0: 1.7) compared to 5 mg/kg bw toltrazuril (mean days oocyst >0: 2.1). Diarrhoea was however not completely suppressed in both treatment groups. Therefore, performance of 10 mg/kg bw toltrazuril was compared to 20 mg/kg bw toltrazuril in a second dose confirmation field study.

The objective of the (non-GCP) controlled field efficacy study was to assess the performance of toltrazuril administered intramuscular in a fixed combination at 10 mg/kg and 20 mg/kg bw, compared to a single oral administration of toltrazuril (20 mg/kg bw).

In this study, 143 neonatal piglets that were naturally infected with *C. suis*, were divided in four groups. On D3, all animals were treated with either a commercial iron product only (control group A) or the fixed combination at 10 mg toltrazuril/kg bw (group B), or 20 mg toltrazuril/kg bw (group C). A fourth group (group D) received both toltrazuril as oral solution and a commercial iron product concomitantly.

Based on the primary efficacy criterion, all toltrazuril-treated groups performed better than the untreated control group A (iron only). There was no significant difference between the three treatment groups B, C and D for the whole period of observation. However, arithmetic mean oocyst excretion was significantly lower in group C versus group B during the first period (D 6-14). Also, when evaluating the faecal score (% piglets with FS >2 at least once), group C performed better (though a significant difference was only present during the second period, and the mean NOD diarrhoea was very low in all groups (Mean NOD diarrhoea: group A: 0.70; group C: 0.38; group D: 0.64). Body weight increase in the group treated at 20 mg/kg bw was significantly higher than in the group treated with 10 mg/kg bw. Diarrhoea was not completely suppressed in the animals in any of the treatment groups neither in the group that received treatment according to label, as well as in the group that received toltrazuril as an oral suspension.

Overall, this study demonstrates superiority of a 20 mg/kg bw dose compared to a 10 mg/kg bw dose of toltrazuril, based on a significantly lower arithmetic mean oocyst excretion during the first period, a significantly greater increase in body weight and a significantly better faecal score during the second study period.

Overall conclusion on dose determination:

The outcome of the studies provided are considered sufficient to support the proposed dose of 20 mg/kg bw toltrazuril and 100 mg/kg iron (as gleptoferron complex).

Target animal tolerance

All the excipients are well-known pharmacopoeial materials. The applicant provided literature supporting the safe use of these substances. It is well-known that in piglets receiving parenteral iron dextran, serious hypersensitivity reactions, including life threatening and fatal anaphylactic/anaphylactoid reactions, have been reported and that deaths have been reported in piglets following the administration of parenteral iron injections. The SPC adequately reflects these warnings.

The applicant provided one GLP compliant study on target animal safety (TAS) of the fixed combination product, as well as safety data for the individual active substances in the already authorised products.

The pivotal multicentre TAS study had a parallel study design and was a controlled, randomized, masked target animal safety study that consisted of three treatment groups (1x, 3x, 5x the recommended treatment dose, RTD) and one control group (NaCl plus iron injection). The study was performed in Germany. For this TAS study, 81 healthy piglets, 2 days of age and weighing 0.9 – 3.39 kg were randomly allocated to four study groups. The number of animals is in line with the VICH GL43 on Target animal safety: pharmaceuticals. The animals chosen constitute a representative sample of the most sensitive population of the target animal species. The final formulation was used.

The objective of this study was to investigate the safety of the investigational product in new-born healthy male (25) and female (29) piglets. According to the VICH GL, individual treatments should be administered for 3 consecutive intervals. The applicant declared that, considering the minimal risk of repeated overdosing, the 5 x RTD group was only treated once, on D3. The 1 x RTD and 3 x RTD groups

were both treated on Day 3, 17 and 31. Therefore, safety was assessed following intramuscular administration at 3 x 1 RTD (20 mg/kg bw toltrazuril + 100 mg/kg bw iron; three times administered, n=12), 3 x 3 RTD (60 mg/kg bw toltrazuril + 300 mg/kg bw iron; three times administered, n=31) and 1 x 5 RTD (100 mg/kg bw toltrazuril + 500 mg/kg bw iron; administered once, n=11). Animals were treated with an intramuscular injection on the third day of life (D3), and animals that belonged to the 1 x RTD and 3 x RTD groups were re-treated on D17 and D31. Animals were not fasted prior to treatment administration; but this can be accepted, considering these were neonatal animals and this accurately reflects the situation in practice where fasting is not deemed appropriate.

This tolerance study did not fully meet the requirements established in the VICH GL43, as an iron negative control group was omitted and there were no repeated 5 x RTD doses administered. However, it is generally agreed practice that new-born piglets should receive iron supplementation, and therefore, omission of an iron–negative control group is considered fully justified from a welfare point of view.

As Baycox Iron is intended to be administered as a single injection, administered only once during a piglet's life, the absence of repeated administrations in the 5 x RTD group can also be accepted for animal welfare reasons. The probability that re-treatment of the same piglet occurs more than 3 times seems very unlikely, and this worst case scenario is covered by the remaining two overdose groups proposed in the TAS study: three applications for the 1 x RTD and 3 x RTD groups.

According to the SPC the dose volume is 0.55 ml/kg body weight. The administration volume was calculated to one decimal place (e.g. 0.1 ml). For this study, the maximum volume to be administered per site was 5.0 ml. According to the dosing instructions of the iron reference product, the animals should receive 200 mg/piglet; however, the applicant administered 91 mg/kg bw. This is accepted, considering that this dose is comparable to the dose administered to animals in the 1 x RTD group.

Total volume administered at each occasion was 0.6 ml/kg bw (1 x RTD), 1.7 ml/kg bw (3 x RTD) and 2.8 ml/kg bw (5 x RTD and control), resulting in overall administration of 60 + 300 mg (1 x RTD), 180 + 900 (3 x RTD) and 100 + 500 mg toltrazuril + iron per kg bw.

The safety of the test item was assessed by daily observations, physical examinations, clinical examinations of the injection sites, body weights, haematology, clinical chemistry, and histopathology, two to three weeks after the last administration of the test and reference item.

Results of the physical examination demonstrated that skin abrasions, biting wounds, lameness, swellings at extremities and claw injuries were observed across all study groups. For a few piglets of the control, 1 x RTD and 5 x RTD groups, no clinical abnormal findings were recorded. The largest number of clinical abnormal findings was found in the 3 x RTD group. Local tolerance was clinically assessed prior to and at 4 hours after injection on the days of injection. In addition, administration sites were assessed one day after injection, once weekly during the 2nd to 7th week of life, and on the day of scheduled necropsy. There were two abnormal findings in the 3 x RTD group following the third injection, a /-6 cm^3 swelling was observed that was 1 cm deep on the right side of the neck and a 0.5 cm^3 swelling on the left femoral musculature in another piglet. Overall, local tolerance at the recommended dose is acceptable.

Haematology and clinical chemistry was also evaluated. Blood was collected about 4 times per group throughout the study. The findings of haematology and clinical chemistry were judged to be of minor clinical relevance.

Nineteen animals had to be removed from the study due to death or disease. Dose (and frequency) relationship in mortality rate has been observed: The 3 x RTD injected intramuscularly three times 14 days apart clearly exceeded the maximum tolerated dose and caused unscheduled mortalities

(n=13/29; 5M, 8F; removal from study between D 34 to 48) related to iron overload (marked systemic deposition of iron, especially in the liver, was observed; and iron related atrophy of lymphatic and hematopoietic organs, secondary infections and organ diseases were considered to have caused mortality). Although the number of deaths that occurred in the 3 x RTD group is somewhat disturbing, the product is expected to only be administered once in an animals' life, and therefore the actual overdose risk is therefore considered to be limited. Also, there were no mortalities following single administration of 5 x RTD.

Abnormal clinical findings and mortality observed in piglets of the 3 x RTD group were considered to be caused by iron. Adverse event recordings in the 1 x RTD group included limb weakness (n=1), sudden death following blood collection and depression (n=1) and increased lung sounds (n=1). Repeated administration of the fixed combination seemed to increase the prevalence of arthritis; and a clear dose-effect could be observed. Arthritis was frequently observed in the 3 x RTD treated groups (n=9; on D5; D18; D10; D20; D32; D18; D13; D21; D32 (most cases developed following the second injection). Arthritis was rarely observed in the other groups and considered not to be related to treatment (control: n=2 (D6; D14); 1 x RTD: n=0; 5 x RTD: n=1x (abscess tarsal joint; D=4)).

At necropsy, 14 days following the last treatment, the 3 x RTD group (both males and females) demonstrated characteristic lesions of bacterial impairment in organs or tissues or septicaemia that were suspected to be a result of the massive iron overload. In this group, clear signs of immunosuppression were observed, and injection site side effects were considerable in this group.

In the 5 x RTD group clay-coloured livers were observed in females and a slight to moderate iron deposition in both males and females was reported. A deposition of iron in the liver was seen once in this group. Other than this, no macroscopic or histopathological findings (including local findings) were observed in this group.

In the 1 x RTD group, injection site side effects in form of inflammation and fibrosis were observed, and a slight to moderate iron deposition in the liver (n=3).

Considering weight of the animals, significant lower body weights in the 3 x RTD group were observed during weeks 4 to 7. In week 7 body weights of the 1 x RTD group were significantly higher compared to the control group. In the 5 x RTD group no statistically significant changes on body weight development were found. Microbiology findings were mostly obtained from 3 x RTD group (8 of 9 animals), and primarily revealed *Staph. aureus* (6 piglets).

According to the applicant, the systemic adverse findings observed in this study were solely related to the iron overload after repeated iron application and not due to toltrazuril, since target animal safety studies of toltrazuril demonstrated safety of the product. This statement is supported by the PSUR reports (2013, 2017) provided for the toltrazuril oral suspension. The applicant also provided scientific literature references on iron metabolism which describe various problems that are known to be associated with iron metabolism, such as an increased susceptibility for infections.

It is agreed that, based on the information available from the PSUR assessments that toltrazuril appears to have a wide margin of safety. Side effects of toltrazuril treatment are very rare in all species concerned, including pigs. In addition, it is well known that iron overload decreases resistance to pathogenic bacteria. Studies have shown that early iron injection can cause health problems such as polyarthritis in piglets and overdose may result in acute toxicity and sudden death.

It can therefore be agreed that the observations of this study are most likely linked to bacterial infections, caused by iron overload.

A sufficiently large difference between 1 x RTD and 3 x RTD (3 x repeated administrations) was present to determine a relevant margin of safety. The product's safety margin appears to be acceptable,

although the number of deaths that occurred in the 3 x RTD group is somewhat disturbing. Results of this study however support the assumption that the use of the product according to SPC would not result in target animal safety issues. These conclusions apply to both local tolerance as well as systemic tolerance. The findings of the target animal safety study are appropriately reflected in section 4.10 of the SPC.

Apart from the target animal safety study, all of the additionally provided studies contribute to the evaluation of target animal safety:

In the two pivotal plasma pharmacokinetic studies, no serious adverse event occurred during the studies, and clinical findings were considered not to be related to the administration of test item. In the two dose determination laboratory studies, as well as the efficacy study, none of the observed adverse events were considered to be caused by the fixed combination product.

In the negatively controlled multicentre field dose confirmation study; in total 58 adverse events (AE) occurred in 57 out of 500 animals (11.40%). Of these, twenty AE were classified as mild and 35 as severe. The local side effects that were observed (n=9; mild AE) are expected to be treatment related. The SPC warnings adequately reflect these potential local side effects.

In the controlled field efficacy study, 16 adverse events occurred in 16 of the 143 animals (11.19%). Three piglets showed discoloration of the skin, the other cases are not considered to be caused by the treatment. The SPC warnings adequately reflect these potential local side effects.

In the negatively controlled multicentre field efficacy study, 86 adverse events were recorded (43 in the test group and 43 in the control group). One adverse event (control group) was classified to be related to the study medication (anaphylactic shock directly following administration of the control product). The SPC warnings adequately reflect this potentially serious adverse event.

In some of the clinical trials that used either the recommended dose (or even less than the recommended dose), leg injuries and/or swollen joints/arthritis were reported in the different groups. Also, mortalities were observed. The applicant adequately addressed these observations. The incidence of leg/joint diseases in the control and treatment groups were similar and below the incidence described in literature (that describes that over 9% of the piglets demonstrate with leg injuries, swollen joints and/or arthritis before nine weeks of age) across all clinical studies with the exception of the 3x treatment group in the target animal safety study. The (treatment-related) increased mortality and arthritis observed in the 3x treatment group has been satisfactorily reflected in Section 4.10 of the SPC.

Though necropsy has not been performed, death of piglets was predominantly related to crushing by the sow or starvation, both conditions are not associated with treatment. Overall, it is agreed that when used as recommended, the combination of toltrazuril and iron injectable suspension is as safe as the treatment with iron only regarding both leg/joint conditions and mortalities.

CVMP considers the safety profile and the margin of safety of Baycox Iron to be similar to that of the single substance products that are already authorised.

Clinical field trials

The applicant provided one pivotal (GCP compliant) field study. The (negatively) controlled, blinded clinical field study was performed in a randomised complete block design. The main objective of this study was to determine the efficacy and safety of the IVP when dosed at 20 mg toltrazuril/kg bw and 100 mg iron/kg bw (test group); in comparison to an untreated (iron only) control group. The study design was adequate. The study was performed in nine different sites located in

Germany, Hungary and Portugal; thus representing different geographical areas and farming conditions in Europe. In total, 968 animals were treated (480 in the test group which received the final formulation at the recommended dose; and 488 animals in the control group).

All animals included derived from farms that had a confirmed history of coccidial infections (primarily *Isospora suis*). In addition, relevant signs of coccidiosis (oocyst shedding and clinical signs of diarrhoea (faecal score >2) during the observation period) were observed in 6 of the 9 farms enrolled.

All animals were treated at three days of age. Animals were weighed prior to dose calculation.

The study did not include a positive control group; and also, for animal welfare reasons, all piglets received an intramuscular iron supplementation at 200 mg iron/animal, which is acceptable. Efficacy of the product against coccidiosis was evaluated as for the dose determination studies:

- Primary evaluation criteria: the "number of days with McMaster countable oocyst excretion >0"
- Co-primary variable: faecal consistency
- Secondary variables: safety parameters (weight gain, laboratory values, safety evaluation).

Results indicated, that in 22.5% of the piglets (30.9% from the control group and 14.0% from the test group), oocysts were detected on at least one day. The treated group had a significantly lower duration of shedding countable oocysts than the negative control group, and a significant lower '*number of days with a faecal score >2'* (test group: 11%, control: 26.2%).

Whilst a statistically significant difference for the parameter 'number of days with McMaster countable oocysts>0' was observed between the treated group and the control group, diarrhoea was not fully prevented in all of the treated animals of the field study (though clinical signs were highly significantly reduced (p>0.001)). However, it is accepted that many confounding factors can contribute to the parameter 'faecal score', specifically in a field situation. Also, efficacy of Baycox Iron and the authorised single substance oral product (20 mg toltrazuril /kg bw) was comparable in preventing diarrhoea in the controlled field efficacy study (see above under dose confirmation studies). The proposed claim, prevention of clinical signs of coccidiosis (such as diarrhoea) in neonatal piglets on farms with a confirmed history of coccidiosis caused by *Cystoisospora suis*, is therefore considered sufficiently supported by the presented data.

Efficacy of the product against iron deficiency anaemia was assessed by comparing laboratory values (haemoglobin, haematocrit, and red blood cells and plasma iron concentration) between the two groups at D3, and D16 (+1). Blood samples were taken from approximately 20% of the piglets.

Additional subgroup analysis, demonstrating haematological values stratified according to three different ranges of body weight (0.9 – 1.2 kg, >1.2 – 1.8 kg and >1.8 kg) were provided.

No statistically significant differences in laboratory values were observed between the different weight groups of animals, nor were significant difference in haemoglobin and bodyweight changes observed between the three different weight categories at D3 and D16. Plasma Iron increased from levels below to levels within the physiological range; and was comparable in both groups (P ≥0.1238).

Overall conclusions on the field study

The applicant conducted one pivotal field study to demonstrate safety and effectiveness of Baycox Iron, representative for the field situation.

Results demonstrate that the product is sufficiently safe, and that the product is considered as effective as the reference products in balancing iron requirements and prevention of clinical signs of coccidiosis

(such as diarrhoea) in neonatal piglets on farms with a confirmed history of coccidiosis caused by *Cystoisospora suis*.

Overall conclusion on efficacy

Justification of the fixed combination:

A satisfactory justification for the combination product has been provided. Thus, the use of this fixed combination versus combined use of single substances will result in a clinical benefit for the animals treated, a practical benefit for the breeder and an improvement of animal welfare.

Pharmacodynamics:

Toltrazuril is a broad-spectrum anticoccidial substance, with well-known efficacy in the prevention of clinical signs of coccidiosis caused by *Cystoisospora suis* in neonatal piglets.

Iron is an essential constituent of haemoglobin and myoglobin and has a key role in enzymes; under intensive farming conditions supplementary iron administration is considered necessary in the first days of life of piglets.

Resistance:

Resistance to toltrazuril is well-known for poultry coccidiosis, and one report has also been provided in *C. suis* in piglets. Therefore, this new combination product must be used only when the indication is well justified. Information on frequent and repeated use of antiprotozoals and preventive measures against anticoccidial resistance development (e.g. the need for hygienic measures) is included in the SPC.

Pharmacokinetics:

After intramuscular administration, the kinetic profile of toltrazuril and its major metabolite, toltrazuril-sulfone (which is also clinically active on all endogenous parasite stages) was comparable to the kinetic profile observed after oral administration, suggesting a comparable clinical efficacy as oral administration of toltrazuril. However, the administration of toltrazuril by intramuscular route showed a delayed absorption than by oral route. Nevertheless, this is not expected to result in differences in efficacy when administered as recommended (i.e. to piglets aged between 48 to 72 hours after birth).

The plasma pharmacokinetics of iron in the fixed combination and an authorised iron monopreparation displayed a very comparable pattern.

Sufficient PK data is provided to conclude on the absence of unintended interactions between both of the two active ingredients of this fixed combination product.

Dose determination:

An oral dose of toltrazuril of 20 mg/kg bw is well-established, and the same dose was proposed for the intramuscular application, based on the pharmacological profile of toltrazuril. Results from a number of dose finding studies testing intramuscular doses ranging 1–20 mg/kg bw confirmed the safety and efficacy of the proposed dose of 20 mg/kg bw, i.m.

The proposed dose of iron (100 mg/kg bw) was based on the doses of products already authorised for intramuscular use for piglets.

Dose confirmation:

Efficacy of the proposed dose of 20 mg/kg bw toltrazuril was confirmed in two controlled dose confirmation trials, one negatively controlled and the other one using a positive control (oral toltrazuril).

Both studies were conducted under field conditions in neonatal piglets in farms with a history of coccidial infection, testing toltrazuril doses of 5, 10 or 20 mg/kg bw.

A single injection of Baycox Iron at 20 mg toltrazuril/kg bodyweight at the 3rd day of life significantly prevented oocyst excretion and highly significantly reduced clinical signs (diarrhoea) associated with porcine coccidiosis (*C. suis*). Comparing secondary endpoints to an untreated control group, piglets treated with Baycox Iron had lower OPG counts, and significantly less piglets excreted oocysts of *Cystoisospora suis*.

A single intramuscular dose of Baycox Iron at Day 3 of age (48–72 hours after birth) proved to be as effective in preventing anaemia in piglets as the positive control product also containing iron as gleptoferron.

Target animal safety:

In the TAS study, a single intramuscular administration of Baycox Iron up to 5x the recommended dose was generally well tolerated in newborn piglets; however, multiple overdoses (3 x RTD) induced clinical signs in some piglets, including apathy, dyspnoea, elevated rectal temperatures, reddening of the skin, ataxia, and/or adverse events of the legs or joints such as polyarthritis or death. These reactions were considered a result of iron overload and are appropriately reflected in the SPC. It is well-known that in piglets receiving parenteral iron dextran, serious hypersensitivity reactions including life threatening and fatal anaphylactic/anaphylactoid reactions have been reported and that deaths have been reported in piglets following administration of parenteral iron. The SPC adequately reflects these warnings.

Toltrazuril is generally considered to be well-tolerated with a wide margin of safety, which is confirmed by PSURs of the single-substance product.

At the recommended dose, Baycox Iron was safe and generally well-tolerated by piglets under field or laboratory conditions. Transient local reactions (discolouration of the tissue and/or slight swelling) at the site of injection were commonly observed. The safety profile of Baycox Iron is similar to that of mono-preparations of toltrazuril or iron at registered doses of veterinary medicinal products containing these active substances.

Clinical field trial:

One pivotal GCP-compliant multicentre European field study was provided, testing the safety and efficacy of a single intramuscular injection of 20 mg toltrazuril/kg bw and 100 mg iron/kg bw in neonatal (3 day old) piglets from farms that had a confirmed history of coccidial infections. Piglets in the control group did not receive any anticoccidial treatment, but did receive routine iron injections.
Results showed in the treated group significantly lower duration of oocyst shedding and number of days with diarrhoea than in the negative control group, and no differences in regard to haematological (iron-related) laboratory values between both groups.

Part 5 – Benefit-risk assessment

Introduction

Baycox Iron is a suspension for injection containing two active substances: 36 mg/ml toltrazuril (an antiprotozoal substance), and 180 mg/ml iron (III) (as gleptoferron; an anti-anaemic substance). The target species is piglets (pigs). The product is available in multi-dose glass vials containing 100 ml.

The applicant applied for the following indication: "*For the prevention of clinical signs of coccidiosis in neonatal piglets on farms with a confirmed history of coccidiosis caused by Cystoisospora suis and concurrent prevention of iron deficiency anaemia in piglets*."

The application has been submitted in accordance with Article 13(b) of Directive 2001/82/EC (fixed combination).

Benefit assessment

Direct therapeutic benefit

Toltrazuril and iron have been authorised and marketed for use in piglets separately for years in the EU as the single-substance veterinary medicinal products. They can be administered separately to the same animal, using the oral and intramuscular routes respectively. Almost 100% of fast-growing piglets need parenteral iron supplementation and coccidiosis is a widespread disease. In many farms therefore, piglets are at risk of developing coccidiosis and consequently these animals need to be treated with iron and a coccidiocide in their first days of life.

Efficacy of this new veterinary medicinal product was demonstrated to be comparable to that of the authorised single-substance reference products containing iron (in preventing iron deficiency anaemia) or toltrazuril (prevention of clinical signs of coccidiosis).

Additional benefits

Neonatal piglets are manipulated very often during the neonatal period (navel cord care, teeth clipping, tail docking, ear notching for identification, castration of males and cross-fostering). Therefore, Baycox Iron presents practical benefits for the breeder, i.e. an injection of iron associated with an oral administration of toltrazuril will be replaced by a single injection of the fixed combination product.

Baycox Iron will facilitate animal handling and improve animal welfare by reducing the number of therapeutic interventions.

Risk assessment

Quality:

Information on the manufacture and controls of both of the active substances have been presented and are satisfactory.

The product's manufacturing process, finished product specification and stability support the authorisation of this medicinal product, and current regulations and guidelines have been taken into account.

Safety:

Measures to manage the risks identified below are included in the risk management section, as described.

Risks for the target animal:

Baycox Iron is considered to be sufficiently safe for the target animal at the recommended dose.

Risk for the user:

Accidental self-injection is shown to be associated with systemic and developmental toxicity effects with regards to the exposure to toltrazuril that requires appropriate warning sentences to mitigate the risks. Other harmful effects identified are related to possible anaphylactoid reactions, local irritancy or granuloma formations that are also addressed with appropriate risk management measures.

Therefore, Baycox Iron is not expected to pose a risk for the user when used according to the SPC.

Risk for the environment:

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

Risk for the consumer:

The residue studies available allow the establishment of a safe withdrawal period of 53 days in pig meat and offal. Therefore, Baycox Iron is not expected to pose a risk for the consumer when used in accordance with this.

Risk management or mitigation measures

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

Evaluation of the benefit-risk balance

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

The applicant applied for the following indication: "For the prevention of clinical signs of coccidiosis in neonatal piglets on farms with a confirmed history of coccidiosis caused by *Cystoisospora suis* and concurrent prevention of iron deficiency anaemia in piglets." The product has been shown to be efficacious in the proposed indications, and the CVMP agreed to the indication but with some slight modifications so it reads as follows: "For the concurrent prevention of clinical signs of coccidiosis (such as diarrhoea) in neonatal piglets on farms with a confirmed history of coccidiosis caused by *Cystoisospora suis,* and prevention of iron deficiency anaemia."

Information on development, manufacture and control of the active substances 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, the environment and consumers, when used as recommended. Appropriate precautionary measures, including the withdrawal period, have been included in the SPC and other product information.

Conclusion

Based on the original and complementary data presented on quality, safety and efficacy, the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for Baycox Iron 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.