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Veterinary Medicines Division

Committee for Medicinal Products for Veterinary Use (CVMP)

Withdrawal assessment report for Aivlosin 42.5 mg/g premix for medicated feed for chickens (EMEA/V/C/000083/X/0081)

International non-proprietary name (INN): Tylvalosin

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

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Introduction

The applicant ECO Animal Health Europe Limited submitted on 31 January 2020 an application for an extension to the marketing authorisation for Aivlosin to the European Medicines Agency (The Agency) in accordance with Article 19 of Commission Regulation (EC) No 1234/2008 and Annex I thereof.

Aivlosin (active substance: tylvalosin), is a macrolide antimicrobial and was first authorised for use in pigs in the Union on 9 September 2004. The product is currently authorised for use in pigs, chickens and turkeys for various antibacterial indications, and is available as premix for medicated feed, oral powder and granules for use in drinking water.

This extension application is for a new target species, chickens, for the 42.5 mg/g premix for medicated feed presentation (currently only authorised for use in pigs). At the time of submission, the applicant applied for the following indication: "Treatment and metaphylaxis of respiratory disease associated with *Mycoplasma gallisepticum* in chickens."

The dossier has been submitted in accordance with Article 19 of Commission Regulation (EC) 1234/2008 and Annex I thereof (extensions).

On 17 March 2021, ECO Animal Health withdrew the application at day 181 of the procedure. In its letter notifying the Agency of the withdrawal of application, the applicant stated that the reason for the withdrawal was based on a commercial decision.

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 (dated 14 June 2019) which fulfils the requirements of Directive 2001/82/EC. Based on the information provided the applicant has the services of a qualified person responsible for pharmacovigilance and the necessary means for the notification of any adverse reaction occurring either in the Community or in a third country.

Manufacturing authorisations and inspection status

Manufacture of the finished product takes place outside the EEA in the United Kingdom. GMP certificates, which confirm the date of the last inspection and show that the sites are authorised for the manufacture of such veterinary dosage forms have been provided for both sites.

Batch release in the EU takes place in Italy.

A GMP declaration for the active substance manufacturing site was provided from the Qualified Person (QP) at the EU batch release site.

Overall conclusions on administrative particulars

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

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

Part 2 - Quality

Aivlosin 42.5 mg/g premix for medicated feeding stuff for chickens is identical to Aivlosin 42.5 mg/g premix for medicated feeding stuff for pigs in all aspects of formulation, manufacture, control testing, type of packaging and pack sizes. Hence a full Part II dossier is not submitted, and reference is made to the Quality data already submitted and approved previously. Only data pertaining to Aivlosin 42.5 mg/g premix for pelleted in broiler feed is provided.

Other Information - In-feed studies

Inclusion rate

The proposed product Aivlosin 42.5 mg/g premix for medicated feeding stuff for chickens has been used to medicate chicken feed at two different inclusion levels (127.5 g and 170 g tylvalosin per tonne feed, corresponding to inclusion rates of 3 kg and 4 kg Aivlosin per tonne feed, respectively). The inclusion rate stated in the proposed SPC is 4 kg Aivlosin per tonne feed.

Homogeneity

Samples from across the bulk blends of two batches of each inclusion rate have been analysed and the results show adequate relative standard deviations (RSDs) of 4.4 to 6.3%.

A risk assessment has been provided that the number of batches of each inclusion rate of 3 kg and 4 kg Aivlosin per tonne are sufficient to ensure that the proposed product Aivlosin 42.5 mg/g premix for medicated feeding stuff for chickens can be homogeneously mixed into chicken feed. Studies have been carried out showing acceptable homogeneity over a wider range of 42.5 ppm to 170 ppm. The 42.5ppm study was able to achieve adequate homogeneity (3.75 %RSD). All mean values were within ± 15% and individual results except one data point (1/100) were within ±25%.

These studies used more than one batch of Aivlosin 42.5 mg/g premix, but only one type of wheat and soybean-based broiler feed has been studied.

Manufacturing procedure

For each batch, a premixture was prepared by mixing either the 3 kg or 4 kg premix with 20 kg of wheat carrier for approximately 2 minutes. The premixture was then added to the mixing vessel with the correct amount of feeding stuff to achieve the desired inclusion rate and the mixture pelleted. The pellets then travel over a sieve where the fine particles are taken off and the pellets are transferred into a 1 tonne metal tote bin for transfer into 20 kg paper bags.

Packaging

Samples were collected into 3-ply Kraft paper bags.

Feeding stuff:

It is considered that the proposed product Aivlosin 42.5 mg/g premix for medicated feeding stuff for chickens can be homogeneously mixed into chicken feed.

Chicken feed is normally pelleted but, should this not be required, it has previously been shown that segregation is not a problem with Aivlosin-containing mash feeds. The stability data provided demonstrated a decreasing trend in the content of assay over time.

Based on the stability data provided a storage time of 3 weeks has been proposed by the applicant. The stability data provided support the proposed storage period of 3 weeks for Aivlosin 42.5mg/g Premix for medicated feeding stuff when mixed into standard broiler feed and pelleted.

Homogeneity and stability have been demonstrated in a variety of wheat, maize and soybean-based feeds. These are the major feedstuff components used in poultry and are common to both broilers and layers. As these components largely determine their physical properties, it is expected that there will be no differences in homogeneity and stability between broiler and layers feed. In addition, two studies in this application were conducted using layers feed and there was no indication of homogeneity or stability issues.

The information provided confirms that there are no significant differences in homogeneity and stability between broiler and layers feed.

Compatibility:

According to the CVMP guideline on medicated premixes (EMEA/CVMP/080/95-FINAL), the compatibility of the active substance with different feed additives usually present in feeding stuffs for the intended target species should be further discussed. If any incompatibilities are known, this information should be added in the product literature. According to the applicant there are no incompatibilities with other veterinary medicinal products known. This is reflected in section 6.2 of the proposed SPC. However, compatibility with feed additives has not been addressed by the applicant. The most commonly used feed additives in chicken feed should be listed and it should be pointed out whether these additives had been present in the feeding stuffs used during stability studies.

However, it should be noted that the stability data provided support at least 3 weeks in use stability of Aivlosin 42.5mg/g Premix for medicated feeding stuff when mixed into standard broiler feed and pelleted. This would be an indication of compatibility of the Aivlosin 42.5mg/g Premix for medicated feeding stuff when mixed into standard broiler feed and pelleted. The information provided is considered acceptable.

Overall conclusion on part 2

Aivlosin 42.5 mg/g premix for medicated feeding stuff has been authorised for pigs. The addition of a new target species chickens makes use of authorised packaging and pack sizes and would therefore not affect the quality of the product. Hence a full part 2 dossier was not submitted, and reference was made to the quality data submitted and approved previously.

Aivlosin 42.5 mg/g premix for medicated feeding stuff has been used to medicate chicken feed at two different inclusion levels. Samples from across the bulk blends have been analysed and the results showed adequate relative standard deviations. Information on the compatibility of the active substance with feed additives usually present in feeding stuffs for the intended target species are still missing and should be provided and included in the SPC section 4.9.

Stability results were variable but clearly showed that pelleted feed was quite unstable but the stability data provided support the proposed storage period of 3 weeks for Aivlosin 42.5mg/g Premix for medicated feeding stuff when mixed into standard broiler feed and pelleted.

Part 3 – Safety

The applicant did not submit any new data for this section of the dossier, instead the application makes reference to the data and previous assessment and conclusions made by the CVMP in the context of two Aivlosin presentations that are already authorised; i.e. 'Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Pigs', which has the same formulation as the product under consideration (and which is the subject of this application to extend the use to a further food producing species), and 'Aivlosin 625 mg/g Granules for use in Drinking Water for Chickens', which is indicated for the same species as the product under consideration.

Safety documentation

Pharmacology and toxicological studies

Since this is an application to extend the marketing authorisation from use in pigs to use in chickens, and the pharmacology and toxicology have already been considered in the MRL applications (EMEA/MRL/794/01-Final, EMEA/CVMP/469245/2007), the initial marketing authorisation and one extension application (Aivlosin, EPAR–Scientific Discussion, November 2009), the applicant has not provided any new data in this section of the dossier. This approach is acceptable. See also part 4.

Development of resistance (food borne bacteria)

See part 4.

User safety

The applicant has not provided a new user risk assessment, and instead refers to the assessment provided previously for the same product intended for use in pigs. It is considered that this is a satisfactory approach, considering the reduced number of occasions in which the product will be administered to chickens in comparison to pigs (7 days consecutive dosing rather than 10 days). The following user safety warnings have been approved for the parent product, and will be retained for use on the product under consideration:

- Tylvalosin has been shown to cause hypersensitivity reactions in laboratory animals; therefore, people with known hypersensitivity to tylvalosin tartrate should avoid contact with the veterinary medicinal product.
- When mixing the veterinary medicinal product and handling the medicated feed, direct contact, with eyes, skin and mucous membranes should be avoided.
- Personal protective equipment consisting of impervious gloves and a half-mask respirator conforming to European Standard EN 149 or a non-disposable respirator conforming to European Standard EN 140, with a filter conforming to European Standard EN 143 should be worn when mixing the veterinary medicinal product.
- Wash contaminated skin.

- In case of accidental ingestion, seek medical advice immediately and show the package leaflet or the label to the physician.

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 proposed SPC.

Environmental risk assessment

The applicant provided an addendum to their environmental risk assessment that was submitted in support of the product 'Aivlosin 625 mg/g Granules for use in Drinking Water for Chickens'. Although the authorised product has an overall dose that is lower than that proposed for the new product under consideration (a total of 119 mg/kg bw vs 75 mg/kg bw), the risk assessment was based on a considerably higher overall dose (150 mg/kg bw) and so the overall outcome of the assessment remains unchanged.

In addition, the assessment of the risk for humans and groundwater ecosystems associated to tylvalosin in groundwater according to the most recent CVMP Guidance (EMA/CVMP/ERA/103555/2015) showed that the use of 'Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Chickens' at a dose of 17 mg/kg bw per day for 7 days does not represent any risk to the environment or to humans (via residues in groundwater when used as drinking water).

The applicant did not provide a specific assessment of persistent, bioaccumulative and toxic (PBT) properties. However, since the $LogK_{ow}$ is < 4 and tylvalosin does not fulfil persistence criteria, it is not considered as a PBT or very persistent and very bioaccumulative (vPvB) substance.

No warnings specific to the environment were included on the already authorised product for pigs, and so it is proposed that there are none added to the labelling for this product either.

In conclusion, the new premix formulation for medicated feed for chickens is not expected to pose a risk for the environment when used according to the proposed SPC.

Residues documentation

MRLs

The active substance in Aivlosin is an allowed substance as described in Table 1 of the Annex to Commission Regulation (EU) No 37/2010:

Pharmacologically active substance	Marker residue	Animal species	MRL	Target tissues	Other provisions	Therapeutic classification
Tylvalosin	Sum of tylvalosin and 3-*O*-acetyltylosin	Porcine	50 µg/kg	Muscle	NO ENTRY	Anti-infectious agents/antibiotics
			50 µg/kg	Skin and fat		
			50 µg/kg	Liver		
			50 µg/kg	Kidney		
		Poultry	50 µg/kg	Skin and fat		

			50 µg/kg	Liver		
	Tylvalosin	Poultry	200 µg/kg	Eggs		

The excipients listed in section 6.1 of the proposed SPC (magnesium trisilicate [sepiolite], wheat flour, hydroxypropyl cellulose and non-fat soybean powder) 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.

Analytical method

The applicant has not submitted new analytical methods for tylvalosin on the grounds that validated and CVMP-approved (see e.g. EMEA/MRL/909/04-FINAL and EMA/CVMP/380628/2014) methods for residue detection in edible tissues from chickens as well as in eggs (YBQ/018) are available and were used for detection of tylvalosin in the frame of the present application. This argumentation is supported.

Pharmacokinetics

The pharmacokinetics of tylvalosin have been appropriately addressed during the establishment of MRLs for poultry (EMEA/CVMP/77339/2005-FINAL). It is agreed that no further information is deemed necessary.

Depletion of residues

Two GLP-compliant tissue depletion studies, one for broiler chickens and one for chicken eggs, were designed as marker residue studies to determine the sum of parent tylvalosin and the metabolite 3-*O*-acetyltylosin concentrations in liver, skin and fat, kidney and muscle of the target animals as well as tylvalosin concentrations in eggs, respectively, to derive withdrawal periods. The broiler chickens were treated with 'Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Chickens' at a target dose of 17 mg/kg bw per day for 7 days orally via feed as intended for marketing. The laying hens in the egg residue depletion study were treated with 'Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Chickens' at a target dose of 20 mg/kg bw per day for 7 days orally via feed, i.e. slightly higher than that intended for marketing. A sufficient number of birds (6/group) and slaughter time points (3, 12, 24, 48 and 72 h after end of treatment) were investigated in the study of chicken broilers. In the study analysing residues eggs, a sufficient number of laying hens (15), eggs (at least 10 per collection time) and collection timepoints (daily from day -1 to day 21, i.e. 1 day before start of treatment and 14 days after end of treatment) were investigated.

Broiler chickens (536–641 g bw on day -1) were treated with actual daily doses of 7.88–29.4 mg/kg bw during 7 days via feed. This is not in accordance with VICH GL 48, which states that '[t]he highest intended dose should be administered for the maximum intended duration'. Tissue residues were determined up to 72 h post dose using a validated LC/MS-MS method (LOQ: 12.5 µg/kg) and corrected for underdosing by using the lowest dose received during day 5 to day 7. The highest marker residue concentrations were observed at 3 h post dose for each type of tissue and depleted rapidly thereafter. Liver was the only target tissue with residue levels above the MRL (50 µg/kg) during the study. The liver marker residue levels were above the MRL at 3 h after end of dosing and below the MRL from 12 h post dose up to the last sampling at 72 h post dose. From 48 h post dose, all marker residue concentrations in the liver were below the LOQ of 12.5 µg/kg. For all

other tissues, including skin with fat (the other target tissue) and the non-target tissues kidney and muscle, the marker residue concentrations were generally below the LOQ in all birds at all timepoints investigated.

Laying hens (1.548–1.846 kg bw on day -1) were treated with actual daily doses of 12.0–27.8 mg/kg bw during 7 days via feed. This is not in accordance with VICH GL 48, which states that '[t]he highest intended dose should be administered for the maximum intended duration'. Residues in eggs were determined up to 14 days post dose using a validated LC/MS-MS method (LOQ: 100 µg/kg). None of the egg samples were found to contain quantifiable tylvalosin concentrations, including those corrected for underdosing by using the lowest dose received during day 5 to day 7. The egg residue concentrations were either below the limit of quantification (100 µg/kg) or below the limit of detection (19.4 µg/kg). From study day -1 to 2 and from study day 13 to 21, all tylvalosin concentrations in eggs were below the limit of detection. The acceptance criteria used for the analysis should be justified.

Withdrawal periods

Chicken

Liver was the only target tissue with residue levels above the MRL (50 µg/kg) in the residue depletion study in broiler chickens. The liver marker residue levels were below the MRL from 12 h post dose and below the LOQ from 48 h to end of dosing. On the basis of these data, 12 h after completion of medication is the first time at which the residues in the target tissues in all birds were below the MRL. Adding a further 12-hour period as a safety span (i.e. 100%) results in a one (1)-day withdrawal period for the use of 'Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Chickens' at a dose of 17 mg of tylvalosin per kg bodyweight per day administered in feed for 7 consecutive days.

Eggs

In the residue depletion study in eggs of chickens presented, no egg from any bird at any time throughout the study contained a tylvalosin concentration above the MRL of 200 µg/kg. The concentrations of tylvalosin were below the LOQ in all egg samples. On the basis of the data generated, a zero (0)-day withdrawal period for eggs following the use of 'Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Chickens' at a dose of 17 mg tylvalosin/kg bodyweight for 7 consecutive days is supported, provided that an acceptable response to the outstanding issue on the acceptance criteria used for the analysis is received.

Overall conclusions on the safety and residues documentation

Safety

The applicant has not provided any (new) data in support of the pharmacological and toxicological part of the dossier, but cross-reference is made to data already submitted and assessed by the CVMP, this is acceptable.

The product is not expected to pose any additional risk to the user when compared to the product currently authorised for use in pigs ('Aivlosin 42.5 mg/g Premix for Medicated Feeding Stuff for Pigs - with the same formulation) when used as recommended.

The premix, when used as recommended, is not expected to pose concerns for the environment when compared to the product currently authorised for use in chickens ('Aivlosin 625 mg/g Granules for use in Drinking Water for Chickens'), where the environmental risk assessment considered a dose

that was almost double that proposed herein. In addition, assessment of the risk for humans and groundwater ecosystems associated to tylvalosin in ground water showed a low risk. Since the $LogK_{ow}$ is < 4 and tylvalosin does not fulfil persistence criteria, it is not considered as a PBT or vPvB substance. In conclusion, there are no outstanding issues for either human or environmental safety.

Residues

Based on the marker residue data and the MRLs established by the CVMP (EMEA/CVMP/77339/2005-Final, EMA/CVMP/380628/2014), withdrawal periods for edible tissues of 1 day for broiler chickens and of 0 days for eggs were calculated and considered acceptable, provided that an acceptable response to the outstanding issue on the acceptance criteria used for the analysis in eggs is received.

Part 4 – Efficacy

This extension application is for a new target species, chickens, for the 42.5 mg/g premix for medicated feed presentation (currently only authorised for use in pigs). At submission, the applicant applied for the following indication: "Treatment of respiratory disease associated with *Mycoplasma gallisepticum* in chickens", which was later amended to add "and metaphylaxis" to the claim. Whilst the applicant did not provide a justification for the indication "metaphylaxis" (as outlined in the CVMP 'Guideline for demonstration of efficacy for veterinary medicinal products containing antimicrobial substances' (EMA/CVMP/627/2001)), a metaphylaxis claim can nevertheless be accepted in addition to a treatment claim, as need for metaphylaxis can be justified for the disease in poultry flocks, and another formulation (Aivlosin granules for use in drinking water) is already authorised for the treatment and metaphylaxis of respiratory infections caused by *Mycoplasma gallisepticum* in chickens.

Pharmacodynamics

The general pharmacodynamic properties of tylvalosin, such as mode and mechanism of action, and spectrum of activity, have been documented in previous applications for Aivlosin granules for use in drinking water for chickens. In addition, three new reports on the antimicrobial susceptibility of *M. gallisepticum* and two studies on tylvalosin bacterial killing kinetics are included in this dossier.

As a brief summary:

- The active substance in Aivlosin is tylvalosin, a macrolide antibiotic that is primarily active against Gram-positive organisms, mycoplasma and some Gram-negative organisms.
- Macrolides interfere with protein synthesis by reversibly binding to the 50S ribosome subunit and thereby inhibiting the protein synthesis.
- Tylvalosin demonstrates both concentration and time dependent killing, although the PK/PD relationship is not fully explored.
- Tylvalosin rapidly enters and is concentrated within cells such as gut epithelial cells and white blood cells.
- Tylvalosin has been shown to be present in relatively high concentrations in respiratory tissue, even when the concentration in plasma is low.
- Macrolides have an effect on the innate immune system. In rodents, pigs and humans tylvalosin has been shown to have an immunostimulating effect on monocytes and macrophages although the clinical relevance of this finding remains uncertain. However, in poultry the effect has been demonstrated to be immunosuppressive. Based on this, the applicant has removed the statement regarding beneficial effects on the immune system from section 5.1 of the proposed SPC.

Regarding the activity against *M. gallisepticum,* reference is made to minimum inhibitory concentrations (MIC) submitted in conjunction with previous procedures in 2006 (submission for granules for use in drinking water for chickens). In addition, three new reports were submitted; one on 21 North American field strains isolated in 1995 and the *M. gallisepticum* R strain from 1985 that was used in the challenge studies; one on 15 UK strains isolated 2008-2012 (9 from chicken); and one on 6 UK strains isolated from chicken in 2016. In addition, 4 isolates were subjected to susceptibility testing in the field trial (Hungary, 2014). Taking both old and newly submitted data into account, tylvalosin MICs ranged from ≤0.001 µg/mL to ≤0.25 µg/ml. Based on a breakpoint for macrolides of 2 µg/mL suggested by Hannan et al., 2000, the applicant concludes that full susceptibility was indicated for all isolates tested. It should however be noted that there are no internationally standardised susceptibility tests for veterinary mycoplasmas or set clinical breakpoints.

The applicant was asked to provide additional MIC data representative for the current situation in the EU. Data for an additional 14 strains isolated from chicken from four European countries was submitted. The tylvalosin MIC for these isolates ranged between 0.016 µg/mL and 0.5 µg/ml. Taking previously submitted data into account, MIC data for a total of 47 strains of *M. gallisepticum* from the target species chicken has been presented. Out of these strains, 20 were isolated within the last five years. Comparing tylvalosin MIC data collected before (n=14) and after 2006 (n=33) there were no indications of any substantial change in the susceptibility profile of *M. gallisepticum* over time. However, it should be noted that conclusions as regards susceptibility trends have to be made with reservations as a comparison of different MIC data is only possible if the methods used for MIC determination are identical. The applicant was asked to describe the methods used for MIC determination for the additional 14 strains submitted.

The amount of MIC data is still considered limited but was accepted taking into account the difficulty in isolating the target pathogen. No additional data indicating tylvalosin susceptibility among tylosin resistant *M. gallisepticum* strains is presented and thus the same information as already included in the SPC and product information for the granules for use in drinking water for chicken presentation was added to section 5.1 of the premix: "Reduced susceptibility for tylvalosin was generally noted in tylosin resistant strains."

The killing kinetics of tylvalosin/*M. gallisepticum* was described in two studies; one including two strains (a reference strain and a Spanish field isolate), and one on the *M. gallisepticum* R strain that was used for challenge. The results from these studies should be interpreted with care since there currently are no internationally accepted standard methods for bacterial killing tests regarding *M. gallisepticum*. For the field strain isolate, the results are not considered to support a bactericidal effect since a sustained, marked drop in viable cell counts corresponding to a -3 log10 change was not detected until 102 h of incubation. For the reference strains, a bactericidal effect was achieved within 48 h at concentrations corresponding to 10x MIC and ≥2x MIC for the two strains, respectively. However, the relevance of the results of time kill experiments for the clinical situation is unclear and inclusion of a claimed bactericidal effect in SPC section 5.1 was not considered sufficiently justified. In response to the questioning of a bactericidal effect the applicant referred to the previously submitted time kill experiments conducted with three strains and also pointed out that MMC (minimal mycoplasmicidal concentration) was determined for six isolates (MIC for these isolates was <0.001 µg/ml and MMC was 0.002 µg/ml for three isolates and 0.004 µg/ml for three isolates). The data presented is still not considered sufficiently robust to be included in 5.1 of the SPC due to the limited number of strains tested.

Development of resistance

Target pathogens

To date there have been no reports of clinical resistance to tylvalosin in *M. gallisepticum,* but it should be noted that data on tylvalosin susceptibility of *M. gallisepticum* is scarce and that set clinical breakpoints for resistance are lacking. Decreased susceptibility of *M. gallisepticum* against other macrolides has been demonstrated in published studies on chicken isolates outside of Europe, and in the study on 22 North American isolates from 1995 submitted by the applicant (two isolates with erythromycin MICs of 16 and >64 µg/ml, respectively). As cross resistance between macrolides is a well-known phenomenon, it seems reasonable to expect that resistance to tylvalosin could also emerge. However, based on the tylvalosin MIC data provided for *M. gallisepticum* isolated from European chickens (n=47) including 20 isolates from 2018-2019 there is as of yet, no indication of any substantial change in the susceptibility profile of *M. gallisepticum.* It should however be noted that conclusions as regards susceptibility trends have to be made with reservations as a comparison of different MIC data is only possible if the methods used for MIC determination are identical. The applicant was asked to determine an epidemiological cut-off value to define the population without any acquired resistance and to perform tests of co- and cross-resistance on isolates with elevated MICs. The applicant referred to the scientific discussion for Aivlosin water soluble granules from 2008 in which *M. gallisepticum* isolates with a MIC>0.06 µg/ml were suggested to be outside the wild-type distribution. Based on the MIC distribution of the additional 33 isolates provided, this cut-off is still considered plausible. It should however be pointed out that the estimated cut-off value is based on a limited number of strains and therefore is somewhat uncertain. No tests for co- and cross-resistance on isolates with elevated MICs were presented. The applicant was asked to justify the omission of this data.

Since macrolides are classified as category C antimicrobials by the CVMP's antimicrobial ad hoc expert group (AMEG) they are considered associated with a higher risk of antimicrobial resistance development than first choice antibiotics in AMEG category D. The following sentence is therefore motivated and should be included in section 4.5 of the SPC: "An antibacterial with a lower risk of antimicrobial resistance selection should be used for first line treatment where susceptibility testing suggests the likely efficacy of this approach".

Food borne and zoonotic bacteria

The applicant focused the discussion on the potential risk for resistance development and transfer of public health concerns from food-born and zoonotic pathogens on *Campylobacter* spp, primarily *C. jejuni* but also *C. coli*, which was considered reasonable by CVMP, although also other species like *Salmonella* spp, *Enterococcus* spp, and *Staphylococcus* spp may be of concern. According to the EU summary reports by EFSA (2020), macrolide resistance in *C. jejuni* isolated from both humans and poultry generally is, and has been, low in the EU, whereas a higher prevalence of resistance in *C. coli* is reported from some countries. The recent emergence of horizontally transferable macrolide resistance in *Campylobacter* (e. g. *erm(B)*-mediated resistance), which often resides on mobile genetic elements that also confer resistance to other key antibiotics, is however of general concern and needs to be closely monitored as a change in resistance pattern may warrant a re-evaluation of the use of macrolides in food-producing animals.

The applicant argues that the authorisation of Aivlosin premix for "treatment and metaphylaxis of respiratory disease associated with *M. gallisepticum*" is unlikely to dramatically increase the overall use of tylvalosin to poultry but may instead result in a shift from the use of Aivlosin for use in drinking water to Aivlosin premix. The CVMP considers it difficult to predict how the authorisation of the premix will impact the total use of tylvalosin in poultry. Moreover, according to the proposed SPC there is a

change to a lower dose, longer treatment duration and increased total dose per treatment course for the in-feed formulation as compared to the water formulation. The applicant was asked to address the consequences of this difference in regard to the risk of resistance development. The applicant argued that Aivlosin premix does not pose a potential difference in risk for resistance development compared to Aivlosin water soluble granules and that the risk for resistance development is low for both formulations. The consequences of the difference in dosing regimen were not directly addressed. The new dosing regimen suggested for Aivlosin premix is considered to constitute a greater risk for resistance development both for the target pathogen and bacteria of human health concern due to the fact that the exposure period is longer and the total dose is higher as compared to the water soluble granules. The questionable support for clinical efficacy of the proposed lower dose of Aivlosin premix as compared to Aivlosin water soluble granules adds to this concern. Taking this into account, the applicant was asked to further justify that the risk for resistance development posed by this product is acceptable.

Pharmacokinetics

The general pharmacokinetic properties of tylvalosin in chicken have been documented in previous applications for Aivlosin 625 mg/g granules for use in drinking water. As a brief summary:

- Tylvalosin tartrate is rapidly absorbed after oral administration and is widely distributed in tissues with the highest concentrations found in the respiratory tissues, bile, intestinal mucosa, spleen, kidney and liver.
- Tylvalosin has been shown to concentrate in phagocytic cells and gut epithelial cells. Concentrations (up to 12 times) were achieved in the cells (intracellular), compared to the extracellular concentration. *In vivo* studies have shown tylvalosin to be present in higher concentrations in the mucous lining of the respiratory and gut tissues compared to the plasma.
- The major metabolite of tylvalosin is 3-acetyltylosin (3-AT), which is also microbiologically active.
- The terminal half-lives for the elimination of tylvalosin and its active metabolite 3-AT range from 1 to 1.45 hours in the chicken.
- Six hours after administration of granules for use in drinking water at a target dose of 30 mg/kg bodyweight for 3 days, the concentration of tylvalosin in the gastrointestinal tract mucosa has a mean concentration of 133 ng/g and in the gastrointestinal contents of 1040 ng/g. The active metabolite 3-AT has a mean concentration of 57.9 ng/g and 441 ng/g, respectively.

In support of the current line extension application, the applicant submitted one new PK study**,** comparing the approved dosing regimen of the granules for use in drinking water with the suggested dosing regimen for the medicated feeding stuff (premix) in broiler chicken. Aivlosin 42.5 mg/g premix for medicating feeding stuff was given via feed at a dose rate of 17 mg tylvalosin/kg bodyweight per day (actual dose 19 mg/kg bw) for up to 7 consecutive days depending on sacrifice times, and Aivlosin 625 mg/g granules for use in drinking water was given via drinking water at a dose rate of 25 mg tylvalosin/kg body weight per day (actual dose 25 mg/kg bw) for up to 3 days depending on sacrifice times. At each sampling point, plasma samples were taken from three chickens and they were then euthanized to allow collection of air sacs tissue (thus only one plasma sample and one air sac sample was taken from each chicken). Concentrations of tylvalosin and the metabolite 3-AT in plasma and air sacs were measured using LC/MS/MS methods (concentration range 5-1200 ng/ml).

Tylvalosin pharmacokinetic (PK) parameters were determined using non-compartment analysis over the complete treatment and sampling period for each Aivlosin dose regimen. Mean values of the three samples from each time point were calculated and used to make a concentration-time curve, from which PK parameters were determined. It should be noted that many plasma samples were below the lower limit of quantification, especially with the premix formulation. It is also noted that the Lower

limit of quantification (LLOQ) value of 5 ng/ml is high compared to the C_{max} value obtained in plasma, and also compared to the MIC values reported. Mean total plasma C_{max} was 38 ng/ml for premix and 34 ng/ml for granules for use in drinking water, and $AUC_{0\text{-}LOQ}$ was 1461 h*ng/ml for premix and 1656 h*ng/ml for the granules. The mean air sac tylvalosin PK parameters were also determined, C_{max} was 225 ng/ml for premix and 201 ng/ml for the granules, and $AUC_{0\text{-}LOQ}$ was 7339 h*ng/ml for premix and 4742 h*ng/ml for granules for use in drinking water.

The applicant concludes that the results demonstrate comparable tylvalosin exposure in plasma and air sacs for both formulations when the whole treatment duration is taken into account. The measured concentration of the active substance in air sacs is however considered to be of limited value, since the relevance of the concentrations in (homogenized) tissue in assessing, if the target tissue exposure was sufficient is very unclear.

Based on the standard deviation shown in the plasma-concentration time curves of this study, the variability is very large. This is not unexpected considering that only one plasma sample is available per chicken and also considering that there is a variability in actual dose given to each chicken. It can however be seen from the plasma-concentration time curve that outlier values have likely affected the results, considering for example the very high plasma concentration observed in the granules for use in drinking water group several hours after removal of the medicated water.

Considering the high variability and low number of samples for each time point, and also considering the fact that the actual dose taken up by each animal is unknown as well as the timing of drug intake in relation to sampling, firm conclusions are difficult to draw from this study and it is not possible to conclude comparable exposure between the two dosage regimens. In addition, the pharmacokinetic profile is different even if the exposure during the entire treatment duration should be similar; for example, the time over a certain concentration may differ. Thus, it is not agreed that similarity in plasma or air sac AUC over the entire dosage period would support similar efficacy considering the distribution pattern for macrolides, the absence of relevant PK/PD index and the doubts regarding the value of concentration data from homogenized air sacs.

Thus, firm conclusions are difficult to draw from this study and it is not possible to reach any conclusions regarding appropriate dose or duration of treatment based on the PK data presented.

While the general pharmacokinetic properties of tylvalosin have been documented in previous applications for Aivlosin granules for use in drinking water for chickens, no valid data are available with the medicated feeding stuff (premix).

The proposed SPC includes the relevant information like relevant metabolites and terminal half-life. However, data regarding concentrations in the gastrointestinal tract were obtained with the granules for use in drinking water and no valid data on tissue concentrations are available with the medicated feeding stuff (premix). This information should therefore not be included in section 5.2 of the SmPC.

Dose justification

The proposed dose of 17 mg tylvalosin per kg bodyweight (bw) per day for 7 consecutive days for the treatment and metaphylaxis of respiratory infections caused by *Mycoplasma gallisepticum* in chickens was established based on the findings of two dose determination studies. The applicant also referred to the pharmacokinetic study presented above.

Dose determination / finding studies

The applicant presented two dose determination studies, one including broilers and one including layers. Both studies were randomised, blinded, negative-controlled, GCP-compliant and were conducted in Colorado, USA. The studies were generally conducted in line with the CVMP 'Guideline for demonstration of efficacy for veterinary medicinal products containing antimicrobial substances' (EMA/CVMP/627/2001). The studies performed in the USA were performed at high altitude. However, one dose confirmation study was conducted in the Netherland which represents a clinical situation in the EU.

Dose determination in broilers

In the dose determination study in broilers (2011), three doses were tested for the treatment and metaphylaxis of respiratory disease associated with *Mycoplasma gallisepticum* (Mg) infection. The investigated dose rates were 8.5 mg/kg bw (actual administered dose 7.4 mg/kg bw), 12.75 mg/kg bw (actual dose 10.9 mg/kg bw), and 17 mg/kg bw (actual dose 14.9 mg/kg bw) administered daily via medicated feed for 7 consecutive days. A negative control group which received no medication was also included. Sixty-four birds were included in each group. A group of 24 birds that were euthanised before experimental infection was also included to confirm the freedom from mycoplasma infection. The study also included birds that were treated preventatively with Aivlosin at different dose levels. Since the applicant makes no claim of the use of the product for prevention of Mg, these results are not discussed.

Birds were challenged at 21 days of age with the R-strain (a virulent strain of Mg), which was also used in all the other challenge studies. The MIC for this strain (0.016 µg/ml) is within the MIC range of European field strains reported by the applicant. Experimental infection induced typical signs of Mg infection (respiratory signs, mainly snicking); however, the mortality rates in the challenge studies performed in broilers were higher than what is generally observed in the field. Treatment with Aivlosin 42.5 mg/g premix in feed was initiated five days after challenge when 18% of the birds showed clinical signs of Mg.

Primary endpoints were combined gross lesions scores (including lesions in trachea, air sacs and peritoneum) and combined clinical observations scores including the six clinical signs couching/snicking, nasal discharge, increased lachrymation, congestion of conjunctival vessels, respiratory rales, and difficulty in breathing. Mortality was a part of the primary endpoint together with clinical observation scores, and it was stated that for efficacy to be demonstrated, mortality should not be higher in the treated group compared to the negative control group. According to the applicant, a statistically significant difference between treated groups and controls for only one of the two primary endpoints was sufficient to conclude on a positive outcome of the study. (Note: The same approach was applied in the two dose confirmation studies). However, this approach, including two primary variables of which only one must be fulfilled to define treatment success, is not appropriate without controlling the type I-error. Furthermore, the fact that one of the primary endpoints consisted of two co-primary endpoints (clinical score and mortality) is an additional concern, although it is acknowledged that the applicant's criteria for success were fulfilled for both primary variables (and both co-primary endpoints) in all the three studies.

Results showed that there was a statistically significant difference between all treated groups compared to controls for the two primary variables, for all dose groups, but the differences were small, and the clinical relevance is questioned. Further, one-sided statistical tests were used for the primary variable which is not acceptable. On request, the applicant presented two-sided p-values. In the highest dose group (intended dose 17 mg/kg bw, actual dose 14.9 mg/kg bw) the mean combined gross lesions scores, 11 days after treatment ended, was 6.7 compared to 8.5 in the

negative controls (score range 0–15). The clinical relevance of a reduction of 1.8 scores is questionable though. The combined clinical observation score, in the period one day after start of treatment to 11 days after treatment ended, was 0.2 in the highest dose group compared to 0.6 in the controls (score range 0–6). A substantial relative reduction in the clinical scores in treated animals as compared to placebo is acknowledged. However, given the low clinical scores noted in both groups, the clinical relevance of the observed reduction is questioned.

A clinically relevant reduction in mortality between treated birds and controls was demonstrated (in the highest dose group overall mortality was 5% and Mg-related mortality was 0%, compared to 27.5% and 10.9% in controls), but high mortality rates are not a typical feature of natural Mg infection. There was a difference between the highest dose group compared to the lowest dose group regarding clinical observations scores (primary variable) and gross lesions scores evaluated at the day after treatment ended (secondary variable) and Mg levels in the larynx measured by qPCR at the end of the study (secondary variable). These results indicate a tendency of increased efficacy with increasing dose but the differences between dose groups were small, and superiority of the highest dose rate was not evident. Further, no clear plateau in response was demonstrated and the effect was limited, even in the highest dose group. It is also noted that no significant difference in Mycoplasma culture results were noted between treated birds and controls. Higher doses than the proposed dose of 17 mg/kg bw were not investigated, and the actual dose taken up by the birds in this group was not more than 14.9 mg/kg bw.

Dose determination in layers

In the dose determination study in layers (2018), three doses were tested for the treatment and metaphylaxis of respiratory disease, with regard to the associated decrease in egg production in laying hens experimentally infected with *Mycoplasma synoviae* (Ms) and *Mycoplasma gallisepticum* (Mg). The investigated dose rates were 4.25 mg/kg bw (actual dose 3.6 mg/kg bw), 8.5 mg/kg bw (actual dose 7.3 mg/kg bw), and 17 mg/kg bw (actual dose 14.2 mg/kg bw) administered daily for 7 consecutive days. A negative control group which received no medication was also included.

Due to deficiencies in study design and weak outcome, this study did not provide support for treatment and metaphylaxis according to the proposed indication in layers. No field study was performed to confirm the efficacy in layers. However, for the Aivlosin 625 mg/g granules for use in drinking water, CVMP previously accepted that clinical efficacy could be extrapolated from broilers to layers. In the light of this, and provided that the applicant answers satisfactory to the remaining outstanding issues related to efficacy in broilers, "chickens" could be accepted as the target species, despite lack of conclusive efficacy data for layers in the current application.

In conclusion, the two presented dose finding studies provide only weak support for the selected dose of 17 mg/kg bw in 3 week old broilers as well as in layers. Higher doses than 17 mg/kg bw (intended dose) were not investigated, and the actual mean doses ingested by the animals were not higher than 14.9 mg/kg bw and 14.2 mg/kg bw.

Dose confirmation studies

The applicant presented two dose confirmation challenge studies in broilers, one was conducted in Colorado, USA and the pivotal European study was performed in the Netherlands. Both studies were blinded, randomised, negative controlled, and GCP-compliant. The studies were generally conducted in line with the CVMP 'Guideline for demonstration of efficacy for veterinary medicinal products containing antimicrobial substances' (EMA/CVMP/627/2001).

In the pivotal EU dose confirmation study (2017), birds were challenged at 21 days of age. Treatment

was initiated four days later when 40% of the birds showed clinical signs of disease. A group of 12 birds were euthanised before challenge to confirm freedom from Mycoplasma infection. Aivlosin 42.5 mg/g premix was administered in feed at an intended dose rate of 17 mg/kg bw daily for 7 days to 120 birds. The actual administered mean dose was 13.2 mg/kg bw. A negative control group of 120 birds received unmedicated feed.

Primary endpoints were gross air sac lesions scores and combined clinical observations scores (including couching/snicking, nasal discharge, ocular discharge/increased lacrimation, conjunctivitis, tracheal rales, and dyspnoea). Mortality was a part of the primary endpoint together with clinical observation scores, and it was stated that for efficacy to be demonstrated, mortality should not be higher in the treated group compared to the negative control group. It would have been appropriate to also evaluate lesions in the trachea, but the applicant explained that this was not feasible as it required trained staff who was not available at the study site.

The choice of statistical analysis model is to an extent data driven. However, the element of data driven decisions seems not to be a concern for the interpretation of the results when more results and details on the process of the statistical analysis has been provided.

The study ended 12 days after challenge, which was two days after the last day of treatment (in comparison, in the other two challenge studies in broilers, the follow up period was 11 days after treatment ended). At the end of the study, the mean air sac lesions score was 6.0 in the treated group compared to 7.1 in the controls (score range 0–12, $p<0.01$). A mean lesion score of 6 in the treated group suggests that treatment did not reduce these lesions to a great extent, and the clinical relevance of a reduction of approximately 1 score is questioned.

The mean combined clinical observation score, in the period from the day after treatment start until the study end, was 0.56 in the treated birds compared to 1.3 in the controls (score range 0–6, $p<0.01$). Although the mean reduction of 0.74 appears to be a modest reduction, it was noted that at the end of the study, 45% of animals in the treated group had no clinical signs of Mg, compared to 7.9% in controls, which reflects a clear effect of treatment. Considering that all birds were challenged at the same time with a virulent strain of Mg, a mean score of 1.3 in the controls appeared low. However, it was accepted that birds in the field often only display one clinical sign of disease (and would therefore be scored 1). Further, it was noted that the majority (78–92%) of birds in the control group displayed clinical signs of disease (a score of 1 or higher).

Mortality due to Mg was 10% in treated birds and 23% in controls ($p=0.01$), which reflects a clear treatment effect. However, the mortality due to Mg, in both the controls and treated animals, is surprisingly high. The high mortality in the treated birds could indicate insufficient efficacy of the product. The results show that death occurred throughout the study period and not just in the beginning of treatment. The causes of death were determined based on lesions only and occurrence of concomitant disease was not investigated. Therefore, other causes than Mg infection, contributing to the high mortality rate, could not be excluded.

The study ended only two days after the last day of treatment, which precludes an appropriate evaluation of potential relapse. Still, even in this short time period, there were signs of relapse. At the end of treatment 70 (65%) of treated birds had no clinical signs of Mg. Of the remaining 37 birds, 35 had a clinical score of 1. Two days later, at the end of the study 48 (45%) of treated birds had no clinical signs of Mg infection, of the remaining 59 birds, 24 birds had a clinical score of 1, 33 birds a score of 2, and 2 birds a score of 3. Hence, it appeared as if a significant proportion of birds, that did not display any clinical signs of Mg at the end of treatment, displayed clinical signs two days after treatment ended. This observation could indicate insufficient exposure to the active substance and suggests that a target dose of 17 mg/kg bw could result in insufficient treatment response. The

applicant acknowledged that disease continued to be progressing after end of treatment but did not provided a plausible reason for the increase in clinical signs that could confute that this finding was actually a sign of insufficient treatment effect. The signs of relapse in the present study could indicate suboptimal dosing which remains a major concern.

In the USA dose confirmation study (2011), birds were challenged with Mg at 21 days of age. Treatment was initiated five days later, when 28% of the birds showed clinical signs of disease. A group of 24 birds were euthanised before challenge to confirm freedom from Mycoplasma infection. Aivlosin 42.5 mg/g premix was administered in feed at an intended dose rate of 17 mg/kg bw tylvalosin daily for 7 days to 144 birds. The study ended 11 days after last day of treatment. The actual mean dose administered was 18.3 mg/kg bw. A negative control group of 144 birds received unmedicated feed.

Primary endpoints were combined lesions scores (including lesions in trachea, air sacs, and peritoneum) and combined clinical observations scores (couching/snicking, nasal discharge, increased lachrymation, congestion of conjunctival vessels, respiratory rales, difficulty in breathing). Mortality was a part of the primary endpoint together with clinical observation scores, and it was stated that for efficacy to be demonstrated, mortality should not be higher in the treated group compared to the negative control group.

Eleven days after treatment ended, a significantly ($p<0.0001$) lower combined lesions score was noted in treated birds (2.8) compared to the negative controls (7.0). A reduction of 4.2 scores on a range from 0–15 reflects a clear treatment effect under the experimental setting. The combined clinical observation score, from one day after treatment started to 11 days after treatment ended, was 0.22 in the treated birds compared to 1.18 in the controls (score range 0–6), and thus a treatment effect was also noted in this regard. The mortality rate due to Mg was 6.3% in the control group compared to 0% in the treated group, which was a statistically significant reduction considered to be of relevance. Antibody levels and Mg levels measured by qPCR were also lower in treated birds compared to controls. Mycoplasma was not cultured after challenge. Treatment is not expected to eliminate the pathogen which is reflected in the proposed SPC.

The study provides support for a treatment effect under experimental conditions, as regards to lesion scores, clinical signs, and mortality, of Aivlosin 42.5 mg/g premix given at the recommended treatment dose of 17 mg/kg bw (the mean dose taken up by the birds was approximately 18 mg/kg bw) daily for 7 days. It is however difficult to determine how the results from this and the other challenge studies would correspond to the clinical situation given that several epidemiological factors influence disease characteristics and treatment outcome during a disease outbreak.

Overall, although the two dose confirmation studies appear to provide some support for efficacy under experimental conditions, obvious signs of insufficient treatment effect were observed in the dose confirmation study performed in the Netherlands. Further, although challenge studies provide a good way to study effect of treatment under controlled conditions, they are not fully representative of natural field Mg infection, as all birds are infected at the same time in a challenge study, and the nature of disease caused by natural Mg infection is complicated by other factors such as vaccination and concomitant diseases. From the data presented, efficacy of the proposed dose 17 mg/kg bw per day for 7 days is not sufficiently supported by data from the dose confirmation studies. Efficacy should be confirmed by satisfactory data from the field.

Target animal tolerance

Target animal safety was evaluated in two studies in broiler chickens and one study in laying hens. One of the studies in broilers was already submitted and assessed by the CVMP previously (in procedure EMEA/V/C/083/X/026).

Safety in broilers

One study was performed with Aivlosin granules for use in drinking water and was previously submitted. The GLP study was performed in 2005 and has previously been assessed to support that Aivlosin granules for use in drinking water are safe in 21-day-old broiler chickens at 6x the proposed dose (6x 25 mg/kg) administered for 5 consecutive days and at 1x the dose for 15 days (5x the proposed dose duration), indicating a wide safety margin of the product.

The pivotal TAS-study in broilers was performed in the UK, using the final formulation of Aivlosin Premix. The GLP study was conducted in 2011 in line with VICH guideline 43 (Target animal safety). Forty 21-day old broilers were divided into 4 groups (10 animals per group). The actual mean doses tested were 0x, 0.98x, 2.8x and 5.0x the proposed dose for 3x the proposed treatment time (proposed dosing regimen=17 mg/kg bw daily for 7 days). The administration of Aivlosin had no negative effect on food consumption, water consumption, or bodyweight in any dose group. The clinical finding "loose excreta" was registered in 5 out of 30 treated birds (1 in the 1x dose group, 1 in the 3x dose group, and 3 in the 5x dose group) but also in 1 out of 10 untreated birds and thus there was no clear association to product exposure. No significant differences were detected between treatment groups with regards to haematology. Two clinical parameters, glucose and uric acid, were statistically significantly different in treated birds compared to the control group but there was no clear dose relationship and the values were within expected biological variability. In the highest dose group animals had an increase in heart weight ($p=0.089$) and a decrease in spleen weight ($p=0.023$). This was however not associated with any relevant abnormal findings on gross or histopathology. Thus, the study is considered to support that Aivlosin premix is safe for 21-day-old chickens when administered at the proposed dose regimen and that the safety margin is reassuring. As chickens which are younger than 3 weeks may sometimes also be treated the applicant was asked to justify the safety margin for younger animals. The applicant pointed out that Aivlosin water soluble granules is authorised for 1-day old chickens and thus, use of the premix for which the recommended dose is lower is also expected to be safe. This conclusion was endorsed.

Safety in layers

A GLP-study in layers was performed in 2016 to evaluate the effect of Aivlosin premix on egg production and egg quality. The study included 24 laying chickens at 36 weeks of age divided into a treatment group administered 20 mg tylvalosin/kg bw for 7 days and an untreated control group. Animals were monitored for 21 days after treatment initiation and eggs collected twice daily. The mean dose achieved was 20.8 mg/kg bw per day corresponding to 1.2x the proposed dose rate.

There were no general health abnormalities recorded during the study. One bird in the control group stopped producing eggs at Day 10. For the other birds, egg production was comparable between the groups (on average one egg per bird per day) but the study had low power to detect small differences in production due to the limited number of birds included. Egg height, egg weight and height: width ratio remained similar and stable across the study duration for both groups. All eggs during the study were classified as sound except from one egg from the treated group at Day 4 that was characterised as a "leaker". Outcomes for the additional parameters (egg thickness, egg strength, and Haugh units) were also comparable between the groups. Overall, administration of Aivlosin at 1.2x the recommended dose had no detrimental effects on egg production or egg quality when administered to layers for 7 days. The applicant was asked to justify why multiplies of the recommended dose and

treatment duration were not included, as is recommended in VICH GL43 (target animal safety). The applicant argued that adult birds are expected to have at least the same level of tolerance to tylvalosin as broilers, with respect to systemic toxicity and that this study was conducted to specifically study the effect on the functioning of the oviduct sine this was considered the key difference between the adult layer and the young broiler. This was accepted.

The safety of the product was also evaluated in five clinical efficacy studies including a total of 2084 treated broiler chickens of 3-4 weeks of age and 720 treated layer chickens of 26 weeks of age. No adverse events were reported in these studies, but it is noted that all abnormal health events considered to be related to production were excluded. The applicant was asked to summarise all the abnormal health events observed in the clinical studies to allow for a comparison between treated and untreated animals. The requested data was provided. No safety concerns associated with Aivlosin premix treatment were indicated.

Clinical field trials

One natural infection field trial in broilers was presented by the applicant. The study was a blinded, randomised, negative controlled, single site, GCP-compliant study conducted in Hungary in 2014. The study was not multicentric (as recommended in the guideline) but was otherwise performed in line with the CVMP 'Guideline for demonstration of efficacy for veterinary medicinal products containing antimicrobial substances' (EMA/CVMP/627/2001).

Three thousand chickens (commercial broilers Ross 308) were included in the study. The birds were derived from a parent flock that was positive for Mg but negative for Ms. Chickens were housed in a broiler shed specifically designed for the study. Considering that the study was a single site study, and not performed at a commercial broiler farm, the applicant was asked to justify that the conditions of the study were representative of commercial broilers farms in the EU. The applicant explained that the main difference compared to commercial broiler farms was that birds were housed in different pens which was accepted for study design purposes. Birds were vaccinated according to a commonly used vaccination scheme in the EU, stock density was within EU requirements, and animals were fed commercial feed. Conditions of broiler farms in the EU vary, but it was accepted that the conditions of the study were sufficiently representative of commercial broiler farms across the EU.

Before the start of treatment, tracheal swabs for culture were taken from 20 affected animals and four samples were positive for Mg. Considering that the parent flock was positive for Mg and negative for *Mycoplasma synoviae*, that birds displayed clinical signs typical, although not specific for Mg infection, at an age when clinical signs normally appear, and that tylvalosin at a dose of 28 mg/kg bw for seven days appeared to be an effective treatment (see below), Mg was a considered to be major pathogen in the disease outbreak. Sampling for other pathogens than Mycoplasma was not performed and co-infections with other pathogens were not excluded.

Treatment was initiated when 3% of the birds showed clinical signs of Mg infection, which occurred when the birds were 21 days old. This design is considered to support a treatment and metaphylaxis claim. Half (1500) of the birds were treated with Aivlosin 42.5 mg/g premix at the intended daily dose rate of 17 mg/kg bw tylvalosin for seven days (actual dose taken up by the birds was 28 mg/kg bw). The remaining 1500 birds (controls) received unmedicated feed. The primary variable was combined gross lesions scores (including lesions in trachea, air sacs and peritoneum, score range 0–14) evaluated in 300 treated birds and 300 controls, 8–9 days after treatment ended (feed consumption was recorded for the remaining birds for an additional 8 days until the study ended). Total lesion scores were categorized into eight categories: scores of 0, 1, 2, 3, 4, 5, 6, and > 6, and effectiveness according to the applicant, was demonstrated if the odds of a chicken in the Aivlosin group having a

particular, or lower, total lesion score (any of 0, ≤1, ≤2, ≤3, ≤4, ≤5, ≤6), was statistically significantly improved compared to the odds of a chicken in the negative control group having a similar score. Mortality, clinical signs, body weight, weight gain, feed consumption, feed conversation ratio, and Mg serology were evaluated as secondary variables.

The body weight gain prior to medication was less than the breed standard but the feed intake was close to normal. The feed consumption during the treatment period was therefore higher than anticipated based on the body weight, which resulted in an actual administered mean dose rate of 28 mg/kg bw tylvalosin. The model-based estimated odds of a chicken in the treated group having a total lesion score (either 0, 1, 2, 3, 4, 5, 6) or a smaller score (if possible), was 33.1 times higher ($P < 0.0001$) than the odds of a chicken in the negative control group having a similar score (primary analysis). Additional analyses showed, that there was an estimated 57% chance of a total lesion score of 0 for a chicken in the treated group, compared to a probability of 3.0% in the negative control group, and that the estimated pen mean combined lesion score was 0.7 in the treated birds and 4.3 in the controls.

Overall mortality was 1.7% in treated birds and 6.9% in controls. Frequency of dead birds with macroscopic lesions at necropsy was 0.3% in treated birds compared to 5.7% in controls. A lower percentage of birds in the treated group displayed clinical signs of disease, compared to controls, from the day after initiation of treatment and onwards. The largest difference was observed one week after treatment ended, when 6.3% of treated birds showed clinical signs of disease compared to 28% in controls. The daily weight gain was higher, and the feed conversation ratio was lower in the treated birds compared to controls. At one week after treatment ended, 23% of treated birds were positive for serology compared to 42% of control birds.

Overall, the study was well designed and well conducted, and appears to confirm the efficacy of Aivlosin 42.5 mg/kg premix for the treatment and metaphylaxis of respiratory disease associated with Mg. A clinically relevant reduction in lesions scores, clinical signs, and mortality associated with natural Mg infection was demonstrated. However, support for efficacy of the proposed dose cannot be gained from this study in account of the fact that the actual dose taken up by the birds was 28 mg/kg bw, i.e. 65% higher than the proposed dose. The lack of a field study supporting efficacy at the proposed dose remains a major concern.

Overall conclusion on efficacy

Pharmacodynamics:

The general pharmacodynamic properties of tylvalosin, such as mode and mechanism of action, and spectrum of activity, have been documented in previous applications for Aivlosin granules for use in drinking water for chickens and no new claims are being made for Aivlosin premix for medicated feeding stuff. In addition, three new studies on the antimicrobial susceptibility of *M. gallisepticum* and two studies on tylvalosin bacterial killing kinetics were included in this dossier. Susceptibility data for *M. gallisepticum* was supplemented with MIC data for an additional 14 European strains isolated within the last five years. Based on the tylvalosin MIC data provided for *M. gallisepticum* isolated from European chickens (47 strains in total out of which 20 were collected within the last 5 years) there is as of yet, no indication of any substantial change in the susceptibility profile of *M. gallisepticum*. However, it should be noted that conclusions as regards susceptibility trends have to be made with reservations as a comparison of different MIC data is only possible if the methods used for MIC determination are identical. No tests for co- and cross-resistance on isolates with elevated MICs were presented. The applicant was asked to justify the omission of this data.

Resistance:

It is considered difficult to predict if the authorisation of Aivlosin premix for "treatment and metaphylaxis of respiratory disease associated with *M. gallisepticum*" will result in an increase in the overall use of tylvalosin to poultry or mainly shift the use of Aivlosin for use in drinking water to Aivlosin premix. However, compared to the water medication, the posology differs, as the premix formulation proposes a lower dose, longer treatment duration and increased total dose per treatment course. This new dosing regimen is considered to constitute a greater risk for resistance development both for the target pathogen and bacteria of human health concern. The questionable support for clinical efficacy of the proposed lower dose of Aivlosin premix as compared to Aivlosin water soluble granules adds to this concern. This needs to be addressed further before any final conclusions can be drawn regarding the risk for resistance development in bacteria of concern for the target animal and for human health.

Pharmacokinetics:

The general pharmacokinetic properties of tylvalosin have been documented in previous applications for Aivlosin granules for use in drinking water for chickens. One new PK study was submitted, comparing the approved dosing regimen of the granules for use in drinking water (25 mg/kg bw for 3 days), with the suggested dosing regimen for the medicated feeding stuff (17 mg/kg bw during 7 days) in chicken. The applicant concludes that the results demonstrate comparable tylvalosin exposure in plasma and air sacs for both formulations when the whole treatment duration is taken into account. Considering the high variability and low number of samples for each time point, and also considering the fact that the actual dose taken up by each animal is unknown, as well as the timing of drug intake in relation to sampling, firm conclusions are however difficult to draw from this study and it is not possible to conclude comparable exposure between the two dosage regimens. In addition, it is not agreed that similarity in plasma or air sac AUC over the entire dosage period would support similar efficacy considering he distribution pattern for macrolides, the absence of relevant PK/PD index and the doubts regarding the relevance of concentration data from homogenized air sacs in assessing if the target exposure is sufficient.

Thus, firm conclusions are difficult to draw from this study and it is not possible to draw any conclusions regarding appropriate dose or duration of treatment based on the PK data presented. However, the general pharmacokinetic properties of tylvalosin have been documented in previous applications for Aivlosin granules for use in drinking water for chickens and no additional data is suggested to be included in the SmPC based on this study. Therefore, the issues with the performed study are not further pursued.

Dose determination:

The dose of 17 mg/kg bw per day for 7 days was established in two dose finding studies (one in broilers and one in layers) and supported by two dose confirmation studies performed under experimental conditions. The two presented dose finding studies provide only weak support for the proposed dose of 17 mg/kg bw for 3 week old broilers as well as for layers. In the dose determination study in broilers, no higher mean doses than approximately 15 mg/kg bw (intended dose 17 mg/kg) were investigated. The results indicate a tendency of increased efficacy with increasing dose but the differences between dose groups were small and superiority of the highest dose rate was not evident. Further, no clear plateau in response was demonstrated and the effect was limited, even in the highest dose group. The dose determination study in layers did not provide support for the selected dose for the proposed indication in layers, due to deficiencies in the study design and weak outcome.

Tolerance:

Aivlosin premix for medicated feed stuff was well-tolerated in chicken in doses up to 5x the recommended dose over 3x the recommended treatment duration in one GLP TAS study in broilers. Safety in layers was evaluated in a separate GLP study which demonstrated that administration of 1.2x the recommended dose had no negative effects on egg production or egg quality when administered for 7 days. The safety of the product was also evaluated in five clinical efficacy studies including a total of 2084 treated broiler chickens of 3-4 weeks of age and 720 treated layer chickens of 26 weeks of age. No safety concerns were identified in these studies. Taken together, it was accepted that the presented data support that Aivlosin premix is safe for the target species when used in accordance with the proposed SPC.

Efficacy:

Efficacy of Aivlosin premix in broilers at the proposed dose regimen was investigated in two challenge studies in the USA (dose determination and confirmation), one challenge study in the NL (dose confirmation) and one European placebo-controlled study performed under field conditions. No study was presented to confirm the efficacy in layers. Considering that CVMP previously accepted that clinical efficacy could be extrapolated from broilers to older laying hens, this was accepted.

The dose confirmation study conducted in the USA provided some support that Aivlosin at the recommended dose of 17 mg/kg bw (resulting in a mean actual dose of approximately 18 mg/kg bw) daily for 7 days is effective in the treatment and metaphylaxis of respiratory disease associated with *Mycoplasma gallisepticum* (Mg) under experimental conditions, as clear reductions in lesions scores, respiratory signs, and mortality were demonstrated.

In the dose confirmation study conducted in the Netherlands, a reduction in clinical signs and mortality was demonstrated when Aivlosin was administered at the recommended dose of 17 mg/kg bw (resulting in a mean dose of approximately 13 mg/kg bw). However, the mortality rate was unexpectedly high, even in the treated group, and the reduction in lesions scores was modest. Importantly, there were signs of relapse only two days after the treatment ended. These observations taken together suggests insufficient exposure to the active substance and that the treatment effect when Aivlosin is administered at an intended dose of 17 mg/kg bw is inadequate. It remains open whether an actual ingested dose of 17 mg/kg bw would have been effective.

The results of the field trial demonstrated efficacy of Aivlosin 42.5 mg/kg premix for the treatment and metaphylaxis of respiratory disease associated with Mg. A clinically relevant reduction in lesions scores, clinical signs, and mortality associated with natural Mg infection was demonstrated. However, support for efficacy of the proposed dose cannot be gained from this study considering that although Aivlosin was administered at the recommended dose of 17 mg/kg bw, the actual dose taken up by the birds was 28 mg/kg bw, i.e. considerably (65%) higher than the proposed dose.

The applicant argues that efficacy has been demonstrated in the challenge studies and that there is no reason to believe that the response to treatment in the field, where disease is expected to be less severe, will be less than in the challenge studies. This is not agreed. Since clinical signs are expected to be less severe in the field, the magnitude of effect observed in challenge studies is not directly translatable to the clinical setting. This is particularly important for the observed reduction in mortality, which was the most obvious treatment effect observed in the challenge studies. Further, the situation in a challenge study is very different from the clinical situation where the epidemiological characteristics of the disease, and environmental factors, will impact on the effect of treatment.

Overall, results from the challenge studies brings serious doubt as to whether the proposed dose is adequate to be effective in the field and there are no conclusive field trials available at the suggested

dose level. Taking into account that for formulations administered via feed, variation in intake between animals can be substantial and sick animals often eat less, it is of particular importance to ensure that the proposed dose provide most animals with an effective dose. In conclusion, the proposed dosage regimen (17 mg/kg body weight per day for 7 days) is not considered adequately supported, and efficacy at this dose has not been adequately demonstrated during clinical use of the product.

Part 5 – Benefit-risk assessment

Introduction

Aivlosin (active substance: tylvalosin), is a macrolide antimicrobial for use in pigs, chickens and turkeys for various antibacterial indications, and is available as premix for medicated feed, oral powder and granules for use in drinking water. This extension application is for a new target species, chickens, for the 42.5 mg/g premix for medicated feed presentation (currently only authorised for use in pigs). At the time of submission, the applicant applied for the following indication: "Treatment of respiratory disease associated with *Mycoplasma gallisepticum* in chickens." This claim was amended later to include the (treatment and) metaphylaxis of respiratory infections.

The dossier has been submitted in accordance with Article 19 of Commission Regulation (EC) 1234/2008 and Annex I thereof (extensions).

Benefit assessment

Direct therapeutic benefit

The proposed benefit of Aivlosin 42.5 mg/g premix for medicated feed is its efficacy in treatment and metaphylaxis of respiratory infections caused by *Mycoplasma gallisepticum* in chickens, which was investigated in a number of well-designed laboratory studies and in one well-designed field study. However, major concerns remain about the correct dose and efficacy during clinical use of the product which currently preclude firm conclusions.

Additional benefits

The product increases the range of available treatment possibilities against respiratory infections caused by *Mycoplasma gallisepticum* in chickens.

Risk assessment

Quality:

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use. Moreover, compatibility of the active substance with feed additives usually present in feeding stuffs for the intended target species should be discussed.

Safety:

Risks for the target animal:

Aivlosin premix for medicated feeding stuff was well-tolerated by broilers in doses up to 5x the recommended dose for 3x the recommended treatment duration. In layers administration of 1.2x the recommended dose had no detrimental effects on egg production or egg quality when administered for 7 days. Safety of the product for the target animal was also supported by the clinical efficacy studies (challenge studies and field trial). The risk for the target animal is considered acceptable when used according to the proposed SPC.

Risk for the user:

The CVMP concluded that user safety for this product is acceptable when used according to the proposed SPC recommendations.

Risk for the environment:

The product is not expected to pose a risk for the environment when used according to the proposed SPC recommendations.

Risk for the consumer:

Concerns remain regarding the relevance of the residue depletion study in eggs with respect to the acceptability of the analytical method (which appear not to be in line with the requirements in the current guidelines). No final conclusion can be drawn on consumer safety until this issue has been satisfactorily addressed.

Special risks:

Concerns have been raised relating to the potential for resistance emergence. It is considered difficult to predict if the authorisation of Aivlosin premix for 'treatment and metaphylaxis of respiratory disease associated with *M. gallisepticum* will result in an increase in the overall use of tylvalosin in poultry or mainly shift the use of Aivlosin for use in drinking water to Aivlosin premix. However, according to the proposed SPC, there is a change to a lower dose, longer treatment duration and increased total dose per treatment course for the in-feed formulation as compared to the water formulation. This new dosing regimen is considered to constitute a greater risk for resistance development both for the target pathogen and bacteria of human health concern. The questionable support for clinical efficacy of the proposed lower dose of Aivlosin premix as compared to Aivlosin water soluble granules adds to this concern. This needs to be addressed further before any final conclusions can be drawn regarding the risk for resistance development in bacteria of concern for the target animal and for human health.

Risk management or mitigation measures

Risk management or mitigation measures will be considered pending additional information from the applicant.

Evaluation of the benefit-risk balance

In the presence of major concerns, no conclusions can currently be taken on the benefit-risk balance of the application.

Conclusion

Based on the original data presented on quality, safety and efficacy the Committee for Medicinal Products for Veterinary Use (CVMP) considers that the application for an extension (EMEA/V/C/000083/X/0081) for Aivlosin, to add a new target species – chickens - for the 42.5 mg/g premix for medicated feed presentation, is not approvable at the present time since "major objections" have currently been identified which preclude a recommendation for marketing authorisation.