10 February 2012
EMA/CVMP/504089/2010
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Lasalocid (bovine species)

On 1 February 2012 the European Commission adopted a Regulation[1] establishing maximum residue limits for lasalocid in bovine species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Lasalocid is intended for use in bovine species for the treatment of coccidiosis and is administered orally via the feed.

Lasalocid had maximum residue limits already established[2] for poultry.

Alpharma Belgium BVBA submitted the application for the extension of maximum residue limits to the European Medicines Agency, on 28 July 2010.

Based on the data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 5 May 2011 the establishment of maximum residue limits for lasalocid in bovine species.

Subsequently the Commission recommended on 1 December 2011 that maximum residue limits in bovine species are established. This recommendation was confirmed on 22 December 2011 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 1 February 2012.

[1] Commission Regulation (EU) No 86/2012, O.J. L30, of 02.02.2012

[2] Commission Regulation (EC) No 1353/2007, O.J. L303, of 21.11.2007

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Summary of the scientific discussion for the establishment of MRLs

Substance name:	Lasalocid sodium
Therapeutic class:	Anti-infectious agents/Antibiotics
Procedure number:	EU/10/179/ALP
Applicant:	Alpharma Belgium BVBA
Target species:	Cattle
Intended therapeutic indication:	Coccidiostat
Route (s) of administration:	Oral by feed

1. Introduction

Lasalocid is an antibiotic from the group of carboxylic ionophores and used as the sodium salt (CAS No 25999-20-6). Lasalocid is produced by *Streptomyces lasaliensis* and is a mixture of several closely related homologue substances A, B, C, D, and E. The sum of the lasalocid homologues B, C, D, and E is limited to 10% of the total weight of the active substance, lasalocid. The substance is mainly active against Gram positive microorganisms.

In veterinary medicine lasalocid is used in poultry for the prevention of coccidiosis caused by *Eimeria spp* administered in feed at the dose of 75 to 125 mg/kg for fattening chickens, 90 to 125 mg/kg for turkeys and 90 to 120 mg/kg for pheasants, partridges and quails. In cattle the substance is used for the treatment of coccidiosis in young (non-lactating) cattle at a dose of 1 mg/kg bw for 28 days also administered via the feed**.**

Lasalocid is authorised as a feed additive under Council Directive 70/524/EEC for the prevention of coccidiosis in chickens and turkeys. As a feed additive the substance is given continuously to chickens and turkeys from day 0 up to 16 weeks, at doses of 75 to 125 mg/kg in feed with a withdrawal period of 5 days.

Lasalocid is not used in human medicine.

The CVMP has previously assessed the consumer safety of lasalocid sodium and established an ADI of 2.5 µg/kg/day (i.e. 150 µg/ 60 kg bw person/day).

Currently, lasalocid sodium is included in the Annex of Commission Regulation (EU) No 37/2010 as follows:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Lasalocid	Lasalocid A	Poultry	20 µg/kg 100 µg/kg 100 µg/kg 50 µg/kg 150 µg/kg	Muscle Skin and fat Liver Kidney Eggs	NO ENTRY	Anti-infectious agents/ Antibiotics

2. Scientific risk assessment

2.1. Safety assessment

Lasalocid sodium was previously assessed by the CVMP and a toxicological ADI of 2.5 µg/kg bw, i.e. 150 µg/person was established based on the NOEL of 0.5 mg/kg/day from the 2-year chronic oral toxicity study in rat and the maternal toxicity study in rabbits and applying an uncertainty factor of 200 due to the limited data in respect to neurotoxicity.

Therefore, no further assessment regarding the consumer safety of the substance is required for the purpose of this extension application.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

The behaviour of ^{14}C-labelled lasalocid sodium in cattle was investigated in a GLP-compliant study involving 12 young ruminating beef cattle. The animals received oral capsules containing radiolabelled lasalocid sodium twice daily at a dose rate of 1 mg/kg bw/day for 10 consecutive days. Four animals (2 males, 2 females) were housed in metabolism cages and blood, urine and faeces were sampled at regular intervals up to 168 hours (7 days) after the last dose, when the animals were sacrificed for tissue residue analysis. The remaining 8 cattle were group housed and sacrificed in groups of 4 (2 males, 2 females) at zero and 72 hours after the last dose, respectively. Analysis of plasma samples showed that a plateau was reached approximately 144 hours after administration of the first dose, indicating that a steady-state was achieved. After cessation of treatment, plasma levels declined rapidly. The major route of excretion was via the faeces, with a mean of 74% of the dose administered excreted 1 day after the last administration, and 80% at 7 days after the last administration. In contrast, urinary excretion was very low (0.6% of the dose administered at 7 days withdrawal). Parent lasalocid was the only compound detected in these two matrices.

Liver contained the highest portion of total radioactive residues (3.6, 1.1, and 0.4 µg/kg at 0, 72, and 168 hours after treatment), while residues in muscle were low (less than 0.05 µg/kg). Total residue concentrations declined steadily over time. Apart from parent lasalocid, three components accounting for more than 0.1 µg equivalents/g but all less than 10% total radioactive residues (TRR) were detected in liver at zero and 72 hours withdrawal. Additionally, a number of minor components (less than 0.1 µg equivalents/g) were found, but these could no longer be detected at 72 hours withdrawal. The identity of the metabolites observed was further elucidated using LC-MS analysis. The metabolites found were dihydroxylasalocid, hydroxylasalocid and another metabolite which identity could not be established. In kidney, parent lasalocid was the major residue. A single unknown component was detected in the male kidney sample, which accounted for 0.017 µg equivalents/g (28.82% TRR). The identity of this peak could not be established as following re-analysis at a later time it was not present. Following mass spectral analysis it was considered that this peak may have been an artefact, though it was not possible to conclude this with absolute confidence. In fat, parent lasalocid was the only compound of importance. A single polar unknown component was detected in the male sample which accounted for 0.016 µg equivalents/g (14.09% TRR); once more it was considerate that this peak was an artefact as it is unlikely based on the results for other tissues and excreta that a polar peak would accumulate in fat. Residue levels in muscle were too low for characterisation.

2.2.2. Residue depletion studies

A GLP-compliant tissue residue study was performed. Thirty young cattle aged 2-3 months, with a weight range of 49-162 kg received feed medicated with lasalocid at a dose of 1.05 mg/kg bw for 28 consecutive days. Groups of 6 animals were sacrificed at 12, 24, 72, 120 and 168 hours after

withdrawal of the medicated feed, and tissues were analysed. The analytical method used was a fully validated and specific LC-MS/MS method with a limit of quantification (LOQ) of 5 µg/kg in all tissues, and limits of detection (LOD) of 0.14 µg/kg in muscle and kidney, 0.13 µg/kg in liver and 2.81 µg/kg in fat. Animals were treated individually in this study according to their respective body weights. The mean (± SD) residues of lasalocid A in muscle were 12.58 µg/kg (±9.98) and 13.68 µg/kg (±6.80) at 12 and 24 hours withdrawal. Seventy two and 120 hours after administration of the product, residues of lasalocid A were not detected in muscle. In liver the residues of lasalocid A at 12, 24, 72 and 120 hours after administration of the dose were respectively 1165.06 µg/kg (±300.55), 943.22 µg/kg (±373.38), 101.39 µg/kg (±51.09) and 17.90 µg/kg (±7.80). In fat and kidney, only the samples obtained after 12 and 24 hours after administration had quantifiable residues which were respectively 21.73 µg/kg (±7.91) and 20.14 µg/kg (±14.25) for fat and 22.20 µg/kg (±6.27) and 26.48 µg/kg (±15.98) for kidney.

Establishment of the marker residue

Based on the results of the total radioactive residues and the metabolic profile, lasalocid A was retained as the marker residue. For the calculation of the ratio of marker to total residue, the results of the marker residue concentration and total residue concentration obtained at 0 day withdrawal time were considered. The ratios obtained were: liver 0.131 (0.489/4.047), kidney 0.331 (0.018/0.056) and fat 0.253 (0.027/0.107), respectively. In muscle a ratio of 1 was considered due to the low residue concentration found.

2.2.3. Monitoring or exposure data

No data provided.

2.2.4. Analytical method for monitoring of residues

A study was performed to develop and validate an analytical method for the quantitative determination of lasalocid A in cattle tissues (muscle, kidney, liver and fat). Quantification of lasalocid A was performed with liquid chromatography-tandem mass spectrometry (LC-MS/MS). All analytical experiments were conducted under the principles of GLP, and in line with the recommendations set forth in the Notice to Applicants, Volume 8 of the Rules Governing Medicinal Products in the European Union. The description of the method was presented according to an internationally recognised format. The method was considered validated in accordance with the requirements of Volume 8.

The limit of quantification of the method has been established at 5 µg/kg in muscle, 10 µg/kg in fat, 50 µg/kg in liver and 10 µg/kg in kidney and the limit of detection has been established at 0.14, 0.14, 0.13 and 2.81 µg/kg for muscle, kidney, liver and fat respectively.

2.2.5. Findings of EU or international scientific bodies

The EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed also assessed a product containing lasalocid sodium for use as a feed additive in poultry further to a request from the European Commission to re-evaluate the substance and give advice on its efficacy and safety. The EFSA Scientific Panel concluded that the data provided proved insufficient to give conclusive answers to several of the questions raised. A lowest NOEL of 0.5 mg/kg bw/day was established from a 2-year chronic toxicity study in rats and a maternal toxicity study in rabbits to which applying a safety factor of 100 would lead to an ADI of 5 µg/kg bw/day. However, it was considered that the similarity between the metabolic profiles of lasalocid sodium in the laboratory animals (rat) and chicken had not been thoroughly established and therefore concerns remained of the adequacy of the evaluation of residues in chicken tissues. The liver was established as the target tissue, however, a marker residue could not be established nor MRLs. The European Commission considered that the re-evaluation of lasalocid sodium showed that the conditions laid down in Directive 70/524/EEC were satisfactory for a specific

product containing lasalocid sodium at 15% and that therefore should be authorised for 10 years. Commission Regulation (EC) No 1455/2004 published such considerations. The Regulation was later on ammended by Commission Regulation (EC) No 2037/2005 to take into account the mofidication of the carrier and the MRLs for lasalocid established under Regulation (EEC) No 2377/90 (repealed by Regulation (EC) No 470/2009).

Commission Regulation (EU) No 874/2010, following an opinion from the EFSA, granted a new authorisation for a feed additive containing lasalocid for turkeys up to 16 weeks with a requirement for post-market monitoring to control the possible development of bacterial and/or *Eimeria spp.* resistances. This regulation also takes into account the MRLs as established by Regulation (EU) No 37/2010.

In September 2007 the EFSA produced an opinion on cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a feed additive, the opinion concludes that adverse health effects in consumers resulting from exposure to lasalocid residues in products from animals exposed to feed cross-contaminated even up to a level of 10%, are unlikely.

3. Risk management considerations

3.1. Potential effects on the microorganisms used for industrial food processing

No data were provided. Although lasalocid has microbiological properties, considering that a proposal for the establishment of a MRL for milk has not been made, no data on the effects of the substance on industrial food processing are requested.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

None.

3.3. Elaboration of MRLs

Based on the residue distribution in tissues and residue depletion data MRL values of 100, 20, 10 and 20 µg/kg can be recommended for liver, kidney muscle and fat, respectively.

Calculation of theoretical daily intake of residues:

Tissue	**MRL (µg/kg)**	**Marker/Total Ratio**	**Total Residues (µg/kg)**	**Consumption Factor (kg)**	**Intake (µg)**
Liver	100	0.131	763.36	0.1	76.34
Kidney	20	0.331	60.42	0.05	3.02
Muscle	10	1	10	0.3	3.00
Fat	20	0.253	79.05	0.05	3.95
Total intake (µg)=					**86.31**
% of ADI=					**57.5**

These MRLs would represent 57.5% of the ADI. When adding the theoretical intake from eggs (42.2 µg), the total theoretical residue consumption would be 85.5% of the ADI.

The product is intended for use in young animals and no residue data in milk were provided, therefore the use should be restricted to non lactating animals.

3.4. Considerations on possible extrapolation of MRLs

In line with Article 5 of Regulation (EU) No 470/09, the Committee considered the possibility of extrapolating the MRL recommended for bovine to other food-producing species with a view to ensuring availability of veterinary medicinal products for conditions affecting food producing animals while ensuring a high level of protection of human health.

Extrapolation of MRLs is based on the fact that animal species of the same class exhibit similar patterns of metabolism and residues. When contemplating extrapolating MRLs the CVMP must consider whether the metabolism of the substance in the species/tissue to which extrapolation is proposed is similar to that in the original species/tissue, and in particular whether the established marker residue is produced in the species/tissue to be extrapolated to. In addition the CVMP must consider the consumer exposure to residues that may occur as a result of consumption of the food commodity to which an MRL has been extrapolated. The ratio of marker to total residues is the tool that enables potential total residue levels in a food commodity to be derived from the MRL. As a result of pharmacokinetic differences between species and between tissues/food commodities within species this ratio should ideally be known for each tissue/food commodity of each species in order to allow establishment of MRLs while ensuring consumer safety. However, for minor species it can be considered that limitations due to lack of species specific data will be compensated for by the fact that the exposure of the consumer to residues in minor species is in general limited. At the present time no scientific rationale has been developed that would allow the establishment of safe MRLs in major species without provision of species specific data.

These principles form the basis for the approach described in the Notice to applicants and Guideline - Veterinary medicinal products - Establishment of maximum residue limits (MRLs) for residues of veterinary medicinal products in foodstuffs of animal origin (Volume 8 of the Rules governing medicinal products in the European Union), which states that "*Considering the knowledge on the variation of residue depletion within classes of animals and therefore on the exposure assessment, the risk characterisation should also not differ substantially within an animal class*" and further detailed in the Note for guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin (EMEA/CVMP/187/00-FINAL).

With regard to the extrapolation to minor species the Note for guidance on the risk analysis approach for residues of veterinary medicinal products in food of animal origin allows, in principle, extrapolation of MRLs to minor species, which in this case means an extrapolation to goats, it does specify that there needs to be confirmation that the marker residue occurs in the minor species and that there needs to be a demonstration of the applicability of the analytical method proposed for residue control.

Existing data indicate that the pattern of metabolites seen in rats, poultry and cattle is similar with the predominant metabolite being parent lasalocid. Based on this existing inter-species metabolism data, the assumption can reasonably be made that the parent metabolite will be the predominant metabolite in the goat and consequently it is accepted as the marker residue for goats as well as for cattle. However, no data were provided demonstrating the applicability of the analytical method in goats tissues and milk. Therefore, in the absence of assurance that the analytical method proposed for residue control can be used for monitoring residues in goats tissues and milk the extrapolation of the bovine MRLs for lasalocid to goats cannot be recommended.

In addition, the CVMP noted that lasalocid exhibits different toxicity profiles among different animal species (as other ionophore substances) and therefore its use in other animal species requires specific investigations in each potential target species.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- an ADI of 2.5 µg/kg (i.e. 150 µg/person) was previously established for lasalocid,
- in cattle, an 80% of the dose is excreted unaltered in faeces and urine,
- the metabolic profile of lasalocid in cattle is very similar to chicken and rats,
- lasalocid A was retained as the marker residue,
- the ratios of marker residue to total residue in cattle are 0.131, 0.331, 1 and 0.253 for liver, kidney, muscle and fat respectively,
- the liver was the target tissue for the lasalocid A residues in all the species studied followed by fat (fat+skin in chickens),
- the proposed MRLs for cattle follow the tissue residue distribution,
- the MRLs recommended should allow for a possible MRL for milk to be established at a later stage,
- an analytical method is available for monitoring of residues in cattle tissues in accordance with the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Union.

The Committee recommends the amendment of the entry for lasalocid in table 1 of the Annex to Commission Regulation (EU) No 37/2010 of 22 December 2010, in accordance with the following table:

Pharmaco-logically active substance	**Marker residue**	**Animal species**	**MRLs**	**Target tissues**	**Other provisions**	**Therapeutic classification**
Lasalocid	Lasalocid A	Bovine	10 µg/kg 20 µg/kg 100 µg/kg 20 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption.	Anti-infectious agents/ Antibiotics

4. Background information on the procedure

Submission of the dossier	28 July 2010
Steps taken for assessment of the substance	
Application validated:	10 August 2010
Clock started:	11 August 2010
List of questions adopted:	8 December 2010
Consolidated response to list of questions submitted:	4 February 2011
Clock re-started:	5 February 2011
CVMP opinion adopted:	5 May 2011