

Cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a feed additive¹

Scientific Opinion of the Panel on Contaminants in the Food Chain

(Question N° EFSA-Q-2005-220B)

Adopted on 20 September 2007

PANEL MEMBERS

Jan Alexander, Guðjón Atli Auðunsson, Diane Benford, Andrew Cockburn, Jean-Pierre Cravedi, Eugenia Dogliotti, Alessandro Di Domenico, Maria Luisa Fernández-Cruz, Peter Fürst, Johanna Fink-Gremmels, Corrado Lodovico Galli, Philippe Grandjean, Jadwiga Gzyl, Gerhard Heinemeyer, Niklas Johansson, Antonio Mutti, Josef Schlatter, Rolaf van Leeuwen, Carlos Van Peteghem, Philippe Verger.

SUMMARY

Following a request from the European Commission, the Panel on Contaminants in the Food Chain was asked to deliver a scientific opinion on cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a feed additive.

Lasalocid sodium is a polyether carboxylic ionophore agent that is authorised according to Regulations No (EC) 2430/1999 and 1455/2004 as a coccidiostat for use in chickens for fattening, chickens reared for laying (up to 16 weeks of age) and turkeys (up to 12 weeks of age) with a maximum content of the active ingredient in feed of 125 mg/kg and a withdrawal period of 5 days. Despite the requirements set for feed business operators in Regulation No (EC) 183/2005, it is generally acknowledged that under practical conditions during the production of mixed feeds, a certain percentage of a feed batch remains in the production circuit and these residual amounts can

¹ For citation purposes: Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on Cross-contamination of non-target feedingstuffs by lasalocid authorised for use as a feed additive, *The EFSA Journal* (2007)553, 1-46

contaminate subsequent feed batches. This cross-contamination may result in the exposure of non-target animal species, and hence the potential health risks for non-target animal species as well as the potential residue deposition in foods derived from these non-target animal species have been evaluated.

Signs of intoxication in animals comprising neurological signs (depression, ataxia, paresis, paralysis, muscle tremor) and cardiac effects (inotropy and tachycardia) have been reported in various non-target animal species following accidental exposure, and neurotoxic symptoms without histopathological changes have been described in experimental studies with dogs. These signs of toxicity are consistent with the mode of action of ionophoric polyether coccidiostats, and are comparable to the symptoms observed in the target animal species at doses exceeding the authorised dose. Particularly sensitive are dogs, calves, rabbits, and horses. The Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that accidental ingestion of poultry feed containing the highest authorised level of lasalocid (125 mg/kg feed) may cause intoxications in non-target animal species.

Cross-contamination of feed for non-target animal species at a hypothetical level equal to 10 % (12.5 mg/kg feed) of the maximum authorised concentration of lasalocid in feed for target animal species could result in an intake of 0.6 mg/kg b.w. per day of lasalocid. This level slightly exceeds the overall no observed effect level (NOEL) of 0.5 mg/kg b.w. derived from controlled studies in experimental animals, conducted as a prerequisite for the authorisation of lasalocid as coccidiostat in poultry. The CONTAM Panel concluded that adverse health effects in non-target animals in the event of cross-contamination are unlikely to occur.

Kinetic studies and residue analyses showed that high concentrations of lasalocid can occur in eggs of laying hens and quails. The deposition of residues in eggs corresponds almost linearly to the concentrations in feeds, and model experiments demonstrated that it is likely that the levels in eggs slightly exceed the provisional maximum residue limit (MRL) of 150 µg/kg following exposure of laying hens to feed cross-contaminated at a level of 2 % with feed containing lasalocid at the maximum authorised concentration for the target animal species (2.5 mg/kg feed)

Cross-contamination of feed material for non-target animal species can also result in undesirable residues in livers of ruminants. Model calculations revealed that cross-contamination at a level of 10 % could result in lasalocid residues in liver of 1400 and 2500 µg/kg in sheep and cattle, respectively.

In consideration of these findings, frequent monitoring of feed materials for non-target animal species, especially feeds intended for laying hens, is recommended. Moreover, feedingstuffs for cattle and sheep as well as the livers of these animals should be monitored for the presence of lasalocid residues.

Human exposure estimates based on worst case scenarios indicate that consumption of products from animals exposed to feed cross-contaminated at levels up to 10 % could lead to lasalocid

exposure slightly above the ADI of 5 µg/kg b.w. as established by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel). Given the fact that exposure to lasalocid residues resulting from cross-contamination of feed is likely to be rare, the CONTAM Panel concluded 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 %, is unlikely.

KEYWORDS: lasalocid, cross-contamination, carry-over, coccidiostat, anticoccidial, ionophore, feed additive, occurrence, exposure, animal health, intoxication, human health.

TABLE OF CONTENTS

PANEL MEMBERS	1
BACKGROUND AS PROVIDED BY REQUESTOR	5
1. Cross-contamination	5
2. Legal provisions as regards minimisation of cross-contamination	5
3. Legal provisions as regards the authorisation of coccidiostats (and histomonostats) for use as feed additive	6
4. Unavoidable cross-contamination (under practical conditions)	7
5. Tolerances	8
TERMS OF REFERENCE AS PROVIDED BY REQUESTOR	8
ACKNOWLEDGEMENTS	9
GLOSSARY OF TERMS USED BY THE PANEL IN ITS OPINIONS ON COCCIDIOSTATS	9
ASSESSMENT	11
1. Introduction	11
1.1. Biological activity of lasalocid	13
1.2. Previous evaluations of the toxicity and safety of lasalocid	13
1.3. Cross-contamination of feed batches	15
1.4. Specific data for lasalocid-based feed additive products	17
2. Methods of analysis for lasalocid	18
2.1. Analysis of lasalocid residues in premixes and animal feeds	18
2.2. Analysis of residues of lasalocid in animal products	19
3. Occurrence of lasalocid	20
3.1. Occurrence of lasalocid residues in feed materials for non-target animal species	20
3.2. Occurrence of lasalocid residues in animal products derived from non-target animal species	21
4. Toxicity of lasalocid	23
4.1. Mechanisms of toxicity	23
4.2. Toxicity of lasalocid in target animal species	23
4.3. Toxicity of lasalocid in non-target animal species	24
4.4. Common drug interactions	27
5. Kinetics and tissue distribution	28
5.1. Kinetics of lasalocid in target animal species	28
5.2. Kinetics of lasalocid in non-target animal species	30
6. Risk characterization	33
6.1. Animal health risks in non-target animal species associated with the accidental consumption of feed materials designated for target animal species	33
6.2. Adverse health effects in non-target animal species as a consequence of cross-contamination of feed batches	34
6.3. Residues of lasalocid in foods derived from non-target animal species	34
6.4. Human health risk associated with residues in foods derived from non-target animal species following exposure of these animals to contaminated feed batches	35
CONCLUSIONS	36
RECOMMENDATIONS	37
REFERENCES	38
APPENDIX	42

BACKGROUND AS PROVIDED BY REQUESTOR

1. Cross-contamination

A feed manufacturing company produces a broad range of compound feedingstuffs. Therefore, in the same production line, different compound feedingstuffs have to be manufactured after each other. After the switch over from one product to the following one, traces of the first product remain in the production line and end up in the beginning of the production of the following product. The transfer from one production batch to the following batch is called “carry-over” or “cross-contamination”.

Cross-contamination in purchased premixtures

Purchased premixtures can contain traces of contamination of other substances due to cross-contamination during the production.

Product related cross-contamination

The following properties of the feed additives and premixes also have an important influence on the cross-contamination behaviour:

- adhesive strength – adhesion to walls
- particle size and density (carrier, substance)
- electrostatic properties.

The cross-contamination decreases according to the product being less adhesive and electrostatic.

Establishment related cross-contamination

The design of the dosage, grinding and mixing equipment has an important influence on the level of cross-contamination. Also the transport and storage facilities and conditions are an important factor for cross-contamination

2. Legal provisions as regards minimisation of cross-contamination

Directive No (EC) 95/69²

Council Directive No (EC) 95/69 of 22 December 1995, laying down the conditions and arrangements for approving and registering certain establishments and operating in the animal feed sector, provides in Article 2 and 3, that establishments manufacturing coccidiostats, manufacturing premixtures prepared from coccidiostats, manufacturing compound feedingstuffs containing

² OJ L 332, 30.12.1995, p. 15. As last amended by Council Regulation EC^o No 806/2003 of 14 April 2003 (OJ L 122, 16.5.2003, p. 1)

premixtures prepared from coccidiostats; have to receive approval to exercise these activities. Also intermediaries putting these products into circulation must be approved. The approval is subject to compliance with the minimum conditions laid down in the Annex.

One of these conditions concern the facilities and the equipment and provides that *“the lay-out, design and operation of the facilities and equipment must be as such to minimize the risk of error and permit effective cleaning and maintenance in order to avoid contamination, cross-contamination and any adverse effects generally on the quality of the products.”*

Regulation No (EC) 183/2005³

Regulation No (EC) 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene is applicable from 1 January 2006 onwards and will replace Council Directive No (EC) 95/69.

Article 10 of Regulation No (EC) 183/2005 provides that feed business operators shall ensure that establishments under their control, are approved by the competent area in case these establishments are manufacturing and/or placing on the market coccidiostats and histomonostats, manufacturing and/or placing on the market premixtures prepared using coccidiostats and histomonostats, manufacturing for placing on the market or producing for the exclusive requirements of their holdings, compound feedingstuffs using coccidiostats and histomonostats or premixtures containing coccidiostats and histomonostats.

Annex II to Regulation No (EC) 183/2005 contains requirements for the feed businesses mentioned in previous paragraph. As regards facilities and requirements it is provided under point 2 of Annex II that *“The lay-out, design and construction and size of the facilities and equipment shall:*

(a) permit adequate cleaning and/or disinfection;

(b) be such as to minimize the risk of error and to avoid contamination, cross-contamination and any adverse effects generally on the safety and quality of the products. Machinery coming into contact with feed shall be dried following any wet cleaning process.”

3. Legal provisions as regards the authorisation of coccidiostats (and histomonostats) for use as feed additive

Article 3 of Council Directive No (EEC) 70/524⁴ concerning additives in feedingstuffs provides that no additive may be put into circulation unless a Community authorisation has been granted. This Community authorisation can only be granted if, taking into account the conditions of use, it does

³ OJ L 35, 8.2.2005, p. 1

⁴ OJ L 270, 14.12.1970, p.1

not adversely affect human or animal health or the environment, nor harm the consumer by impairing the characteristics of animal products.

Lasalocid sodium has been authorised for use as feed additive in accordance with the provisions of Council Directive No (EC) 70/524 (see Table 1).

Regulation No (EC) 1831/2003⁵ of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition is applicable since 19 October 2004 and repeals Directive No (EC) 70/524 with effect from that date.

Table 1. Species or category of animals for which the use of lasalocid sodium as feed additive is authorised (target animal), and authorised maximum content in complete feed

Coccidiostat (active substance)	Species or category of animals for which the use of coccidiostats is authorised (target animal)	Authorised maximum content of active substance in complete feed
Lasalocid sodium	Chickens for fattening	125 mg/kg (Avatec)
	Chickens reared for laying (max 16 weeks)	125 mg/kg (Avatec)
	Turkeys (max 12 weeks)	125 mg/kg (Avatec)

4. Unavoidable cross-contamination (under practical conditions)

Lasalocid is authorised for use as a feed additive for the production of feedingstuffs for target animal species according to the conditions of authorisation. However the production of feed containing lasalocid can result in cross-contamination to feedingstuffs for non-target animal species.

Of major importance is the application by the feed operator of good manufacturing practices to avoid to the largest extent possible, the cross-contamination of residues of the coccidiostat in subsequent batches of compound feedingstuffs. However, even if all prevention measures are applied, including the use of rinsing batching, the cross-contamination of residues is unavoidable under practical conditions.

⁵ OJ L 268, 18.10.2003, p. 29–43

5. Tolerances

Therefore, the possibility to set tolerances for these in practice unavoidable residues of coccidiostats in feedingstuffs for non-target animal species should be considered in the frame of Directive No (EC) 2002/32 of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed⁶.

Such tolerances in feedingstuffs for non-target animal species could be set following the ALARA principle (As Low As Reasonably Achievable) taking into account good manufacturing practices. According to information received from professional organisations, a range of 3 - 10 % with a majority at 5 % or lower can be achieved after implementing thorough actions to reduce cross-contamination.

Such tolerances in feedingstuffs for non-target animal species should not have a pharmacological activity and not threaten animal health and public health, as in some cases the tolerances for feedingstuffs for non-target animal species could result in presence of residues in products of animal origin.

TERMS OF REFERENCE AS PROVIDED BY REQUESTOR

In accordance with Article 29 (1) (a) of Regulation No (EC) 178/2002⁷ the Commission asks EFSA to provide an opinion on the risks involved for animal health and public health as the consequence of undesirable cross-contamination of lasalocid sodium authorised as feed additive into non-target feeds.

The assessment should take into account hypothetical cross-contamination rates of 2 %, 5 % and 10 % from feed produced with the highest authorised dose of lasalocid sodium into the afterwards produced non-target compound feed (for non-target animal species).

The EFSA is requested to provide an opinion whereby

- the animal health risk for non-target animal species (food producing farm animals) will be assessed,
- the adverse effects as a consequence of cross-contamination of lasalocid sodium into feed for non-target animal species,
- on the basis of the available information, an estimate of the level of residues present in food of animal origin from non-target animal species as the consequence of cross-contamination is performed,

⁶ OJ L 140, 30.5.2002, p. 10. Directive as amended by Directive No (EC) 2005/6 (OJ L 24, 27.1.2005, p. 33)

⁷ OJ L 31, 1.2.2002, p. 1–24

- the possible risks for human health as the consequence of the presence of such residues in food of animal origin (eggs, milk, meat, edible offal) from non-target animal species are assessed.

ACKNOWLEDGEMENTS

The European Food Safety Authority wishes to thank the members of the Working Group for the preparation of this opinion: Arturo Anadón, Alexander Feil, María Luisa Fernández-Cruz, Johanna Fink-Gremmels, Kimmo Peltonen, Derek Renshaw, Pascal Sanders and Pieter Wester.

GLOSSARY OF TERMS USED BY THE PANEL IN ITS OPINIONS ON COCCIDIOSTATS

Considering the current EU legislation, the following terms will be applied in the Opinion:

Coccidiosis: Coccidiosis is a common protozoan infection in farm animals, affecting predominantly young animals. Under common farm conditions, herd health management cannot exclude coccidial infections in large poultry and rabbit units and the use of coccidiostatic agents (coccidiostats) remains necessary to control animal health and welfare, and to avoid substantial losses due to acute and often lethal coccidiosis.

Coccidiostats: Currently, in the EU 11 coccidiostatic substances are authorised for the prevention of coccidiosis in one or more animal species. Authorisation is given for a minimum and maximum level to be included as feed additive into the animal's diet, and may prescribe the animal species as well as the species categories (age groups) and in some cases withdrawal periods have been set to avoid undesirable residues in edible tissues of treated animals. Of the 40.65 million tonnes of feed produced annually for chickens for fattening, turkeys and rabbits, approximately 18.33 million tonnes is manufactured with the addition of a coccidiostat (IFAH, 2007, document provided to EFSA).

Various coccidiostats also exert a distinct antibacterial effect and are licensed in Third Countries (countries outside the EU) as growth promoting agents in fattening ruminants (lambs or cattle) and fattening pigs.

Target animal species: Animal species or animal category (age group) within a species for which the compound under consideration is licensed for use as a coccidiostat. This term also covers chickens reared for laying or turkeys until the age of 12 or 16 weeks (as defined in the authorisation of the specific product). The choice of either 12 or 16 weeks depends on the request made by the applicant and/or the data submitted. The chicken or turkey thereafter turns into a non-target animal species. A hen starts egg laying between 18 and 26 weeks of age.

Non-target animal species: Any other animal species or category for which the compound is not authorised.

Feed additive: A substance, micro-organism or preparation, other than feed material and premixtures, which are intentionally added to feed at concentrations up to a defined maximum level (mg/kg feed). Currently, coccidiostats are authorised for use as feed additives according to the provisions of Directive No (EC) 70/524 and Regulation No (EC) 1831/2003 that repeals Directive No (EC) 70/524 (see also Terms of Reference). According to these provisions, authorisation and prerequisites for use are defined for individual products (brands) following review by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the data provided by the applicant.

Premixture: Pharmaceutical formulation of a compound (coccidiostat) intended to be mixed with feed. Premixtures are not intended for direct consumption by animals, and are therefore not addressed in the Opinion.

Cross-contamination: Contamination of feeds that are produced after the production of a mixed feed containing additives with residual amounts of the previous feed batch.

Levels of cross-contamination: According to the mandate as described in the Terms of Reference, three levels of cross-contamination will be considered, i.e. 2 %, 5 % and 10 % of the maximum concentration authorised for target animal species, respectively.

Assessment of animal exposure and adverse health effects in animals: Adverse health effects occurring in non-target animal species are described. A distinction is made between the likelihood of adverse health effects that are associated with an accidental consumption of feeds prepared for a target animal species by a non-target animal species, and the involuntary exposure of non-target animal species by residual amounts of coccidiostats occurring in feed as a consequence of cross-contamination.

ADI values: Acceptable daily intake (ADI) of a substance that can be consumed by a human over a lifetime without adverse health effects. As the CONTAM Panel did not have access to the complete safety (toxicological, pharmacological and microbiological) database available for the individual substances under consideration, the ADI value as derived by the FEEDAP Panel and where appropriate also the ADI(s) derived by other relevant scientific committees (e.g. the CVMP⁸ or the JECFA⁹) is used for the risk characterization. The CONTAM Panel noted in some cases the divergence between ADI values derived by the FEEDAP Panel and the ADI values derived by the CVMP and/or JECFA. These differences were attributable to the application of different uncertainty

⁸ The Committee for Medicinal Products for Veterinary Use of the European Medicines Agency

⁹ The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is an international expert scientific committee that is administered jointly by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

factors, or the inclusion of new endpoints, such as antimicrobial activity (antimicrobial no-effect level) in the assessment. The CONTAM Panel decided to consider both values in the presentation of its risk assessment for non-target animal species.

MRL values: Maximum residue limits. The CVMP applied Regulation No (EC) 1055/2006¹⁰ amending the Annexes I and III of Regulation No (EC) 2377/90¹¹ to propose maximum residue limits (MRLs) for a number of coccidiostats. However, none of the compounds under consideration are licensed at present as veterinary medicinal product. The FEEDAP has also recommended MRLs for some coccidiostats.

Residues of coccidiostats in edible tissues, milk and eggs: According to Directive No (EC) 96/23¹² Member States are obliged to monitor certain substances and residues thereof in animals and animal products. These data are collected by the Commission and a compilation of the results from 2004 and 2005 are used in the human exposure assessment.

Human dietary exposure: The present assessment is confined to the evaluation of residues of coccidiostats in foodstuffs derived from non-target animals. Where appropriate, total dietary exposure originating from different products including edible tissues, milk and eggs is estimated.

Risk characterization: The risk characterization is based on the ADI and MRL values from either the FEEDAP Panel, the CVMP or the JECFA as outlined above. These levels are compared with levels of residues found in tissues and/or products (for example eggs) of non-target animal species as far as these are available. Where appropriate uncertainties in the establishment of ADI values are discussed.

ASSESSMENT

1. Introduction

Lasalocid sodium belongs to the divalent polyether ionophore family. Lasalocid monoacid is produced by fermentation of *Streptomyces lasaliensis* subsp *lasaliensis*. The lasalocid monoacid is recovered from the fermentation media by acidification and then extracted. The main active ingredient is lasalocid sodium A representing at least 90 % of the active ingredient in authorised products. The lasalocid homologues B, C, D and E, each corresponding to one ethyl substitution of a methyl group in different positions of the molecule, are present as minor components at a maximum of 10 % of total lasalocid (EFSA, 2004).

The chemical structure of lasalocid is presented in Figure 1.

¹⁰ OJ L 192, 13.7.2006, p. 3–5

¹¹ OJ L 224, 18.8.1990, p. 1–8

¹² OJ L 125, 23.5.1996, p. 10–32

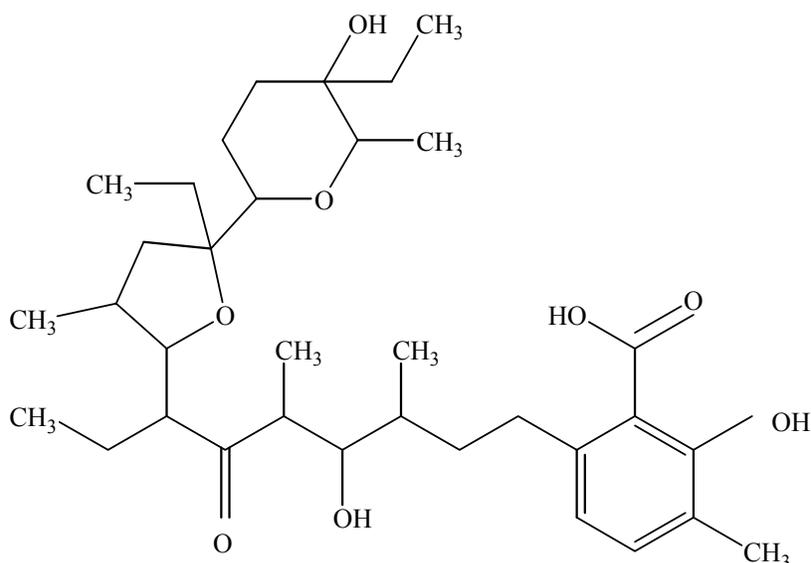


Figure 1. Chemical structure of lasalocid

The IUPAC name of lasalocid is 6-[7-[5-ethyl-5-(5-ethyl-5-hydroxy-6-methyl-oxan-2-yl)-3-methyl-oxolan-2-yl]-4-hydroxy-3,5-dimethyl-6-oxo-nonyl]-2-hydroxy-3-methyl-benzoic acid, the CAS Number 25999-20-6, the molecular weight 590.8 and the molecular formula $C_{34}H_{54}O_8$. Although differences in the molecular structure exists between the monoacid and the sodium salt, the most frequents synonyms of the IUPAC name and CAS Number of lasalocid are lasalocid sodium salt, lasalocid A monosodium salt, and X-537A.

The molecular weight of lasalocid sodium is 612.8 g (sodium included), the water solubility at 30°C is 1.06 g/L and the log octanol/water partition coefficient (K_{ow}) varies between 1.4 and 2.3.

As summarized in the Terms of Reference, lasalocid sodium (Avatec 15 %/150g) is authorized for the control of coccidiosis, in the EU at a minimum-maximum concentration of 75 - 125 mg/kg feed, in chickens for fattening and in chickens reared for laying (up to 16 weeks of age) (Regulation No (EC) 1455/2004¹³) and, at a minimum-maximum concentration of 90 - 125 mg/kg feed, in turkeys (up to 12 weeks of age) (Regulation No (EC) 2430/1999¹⁴). The withdrawal period is five days for both animal species. Lasalocid sodium is registered in the United States of America for use in sheep, cattle, rabbits, chickens and turkeys as anticoccidial and growth promoting agent.

In Third Countries, lasalocid sodium is licensed for use as a growth promoter in sheep, cattle, and rabbits. The licensed feed levels are much lower than those used in the prevention of coccidiosis, as a maximum concentration of 33 mg/kg feed are given to cattle and sheep. For rabbits feed concentrations between 68 - 113 mg/kg are licensed for the treatment of hepatic coccidiosis.

¹³ OJ L 269, 17.8.2004, p. 14

¹⁴ OJ L 296, 17.11.1999, p. 3

1.1. Biological activity of lasalocid

Anticoccidial activity

Lasalocid, like other polyether ionophores, is effective against sporozoites, and early and late asexual stages of coccidia in the intestines of poultry. The biological activity of ionophoric coccidiostats is based on their ability to form lipid soluble and dynamically reversible complexes with cations. Lasalocid sodium, like other polyether ionophore compounds, has different ionic affinities, binding divalent cations such as Ca^{2+} and Mg^{2+} , as well as monovalent ions including Na^+ and K^+ , increasing their passage across biological membranes.

The ionophoric properties of lasalocid lead to the disruption of the normal physiological processes of prokaryotic and eukaryotic cells. Coccidial sporozoites exposed to lasalocid sodium in the intestinal lumen show significant and potentially lethal osmotic damage (EFSA, 2004).

Antibacterial activity

Lasalocid sodium has a selective antibacterial spectrum in a concentration range between 0.06 and 4 mg/L against Gram-positive bacterial species such as *Enterococcus faecium*, *E. faecalis* and *Staphylococcus* spp., while many *Enterobacteriaceae* are naturally resistant. Inhibitory concentrations for susceptible strains are lower than the dose incorporated in feed and antibacterial concentrations are likely to be attained *in vivo*. Induction of resistance and/or cross-resistance was not observed under experimental conditions (EFSA, 2004).

On the basis of various studies with samples of human gut microbiota, a microbiological ADI was calculated by EMEA (EMEA, 2004)¹⁵. This microbiological ADI of 4.91 $\mu\text{g}/\text{kg}$ b.w. is higher than the toxicological ADI (see below), the latter was considered by the EMEA as the overall ADI for the assessment of the safety of consumers.

Lasalocid is not used in human medicine and is not classified as a critically important antibiotic by WHO experts¹⁶. However, it has been listed by the Office Internationale des Epizooties (OIE, World Organization for Animal Health) (OIE, 2006) as a veterinary highly important antibiotic for use in the control of coccidiosis.

1.2. Previous evaluations of the toxicity and safety of lasalocid.

Lasalocid has recently been assessed by the FEEDAP Panel (EFSA, 2004) and the CVMP (EMEA, 2004). Both committees used a comparable data set comprising oral toxicity in mice, rats, neonatal rats, rabbits and dogs (the original data are not available to the CONTAM Panel). According to these reports, the oral median lethal dose (LD_{50}) values were found to vary between 122 and 146

¹⁵ The EMEA, which evaluate veterinary medicines, is required to evaluate the possible effects of microbiologically active substances on the bacterial populations of the human gut flora and to identify a microbiological ADI.

¹⁶ Critically important antibacterial agents for human medicine for risk management strategies of non-human use : report of a WHO working group consultation, 15-18 February 2005, Canberra, Australia.

mg/kg b.w. in adult rats and mice, respectively, whereas neonatal rats and rabbits were found to be more sensitive with oral LD₅₀ values of 33 and 40 mg/kg b.w., respectively (EMEA, 2004). For subchronic toxicity, three 13 week oral studies were performed in Charles River Sprague-Dawley rats (CD rats) at doses of 0, 1, 2, 3 and 10 mg/kg b.w per day showing that female rats are more sensitive to the effects of lasalocid than male rats. Common pathological findings included, among others, decreased haematocrit and haemoglobin, leucocytosis, increased haemosiderin levels in kidneys and liver and elevated transaminases in the blood serum at 3 mg/kg b.w. per day. In addition to these effects, a vacuolisation of cardiac muscle tissue was observed at a dose of 10 mg/kg b.w per day. From these studies a NOEL of 1 mg/kg b.w. per day was derived, based on the occurrence of a slight decrease in haematocrit and a slight neutrophilic leucocytosis in female rats at 2 mg/kg b.w. per day. In addition, a 13-week oral toxicity was conducted in beagle dogs using doses of 0, 2, 5 and 10 mg/kg b.w. per day given in gelatine capsules. In this study a NOEL of 2 mg/kg lasalocid was derived, based on vacuolisation of hepatocytes together with neurological clinical signs but without corresponding histopathological changes.

Single and three-generation reproduction and teratology studies in rats provided no evidence for visceral or skeletal abnormalities, and a NOEL of 0.5 to 0.8 mg lasalocid/kg b.w. was derived from these experiments. In rabbits (oral gavage study) skeletal deformations were observed at high concentrations (see 4.3.6.). The NOEL for foetal toxicity was 0.5 mg/kg b.w. based on maternal toxicity. Lasalocid was also found not to be genotoxic in a battery of *in vitro* assays (EFSA, 2004). In terms of chronic toxicity, studies in rats and dogs have been performed. For the 2-year rat oral toxicity study, doses of 0, 0.5, 1.8 and 6.2 mg/kg b.w. and 0, 0.6, 2.2 and 8.1 mg/kg b.w. per day were administered in the diet to males and females respectively (EFSA, 2004). No adverse effect on survival incidence, blood chemistry parameters, clinical signs, neurological effects, ocular changes or increases in the incidences of apparent tumours and nodules occurred at any dose level. Increases in liver weight in both sexes treated at the mid and high doses group, and in adrenal gland weight in females at the mid and high dose groups were observed. NOEL values of 0.5 and 0.6 mg/kg b.w per day for males and females were derived from these endpoints respectively. In comparison, dogs were less sensitive than rats, and a NOEL of 1 mg/kg b.w. per day was derived for both sexes based on an increase in alkaline phosphatase.

Based on the results of a 2-year chronic rat toxicity study, the NOEL of 0.5 mg/kg b.w per day was used to derive an ADI of 5 µg/kg (0.005 mg/kg) b.w. per day by the FEEDAP Panel by applying a 100-fold uncertainty factor (EFSA, 2004). The CVMP used the same NOEL from the chronic oral toxicity study in rats and a developmental study in rabbits to derive the ADI. However, the CVMP applied an uncertainty factor of 200 due to the limited data regarding neurotoxicity giving an ADI of 2.5 µg/kg (0.0025 mg/kg) b.w. per day.

Additionally, on the basis of a number of studies using samples of human gut microbiota, a microbiological ADI of 4.91 µg/kg (0.00491 mg/kg) b.w. was derived by the CVMP (EMEA, 2004). This level is higher than the toxicological ADI set by the CVMP and similar to the ADI set by the FEEDAP panel.

Although lasalocid is not licensed in any veterinary medicinal products, it has now been included by Regulation No (EC) 1055/2006 in Annex I to Regulation No (EC) 2377/90¹⁷ with MRLs for muscle, skin/fat, liver and kidney of poultry (the target animal species) of 20, 100, 100 and 50 µg/kg, respectively, and in Annex III with a provisional MRL of 150 µg/kg in eggs. The provisional MRL is recommended by CVMP to be established as a full MRL at the same value (EMEA, 2007). These MRLs are all based on assessments by the CVMP (EMEA, 2004, 2006).

1.3. Cross-contamination of feed batches

Feed additives, such as coccidiostats, are marketed as premixes, intended to be incorporated into mixed feeds during the mixing and production process. Cross-contamination refers to the fact that under the practical conditions in a commercial feed mill, residual amounts of feed materials remain in the production line (as outlined in Article 10 of Regulation No (EC) 183/2005; see also Terms of Reference) and may contaminate subsequent feed batches. The degree of cross-contamination depends on the technical facilities and procedures, as well as on product characteristics.

1.3.1. Factors influencing the rate of cross-contamination

Several studies have shown that a completely contamination-free production of premixes and compound feeds in existing multi-product plants is impossible in practice (Strauch, 2003). Various process parameters and physicochemical characteristics of the product act together to determine the residual amount remaining in the circuit and hence the rate of cross-contamination from one feed batch to the subsequent batches produced in the same production line (Kennedy *et al.*, 1996, 1998; McEvoy *et al.*, 2003; Harner *et al.*, 1996). In a similar way, the purchased premix that is incorporated into the feed can itself contain traces of contamination of other substances, due to cross-contamination during the production of the premix.

The **technological equipment** in the feed mill can influence the amount of cross-contamination that may occur. The following sites in the circuit have been identified as being places where fractions of feeds can be retained, with the possible consequence of contamination of later batches:

- Areas of reduced flow in piping, material ledges, and non-plane surfaces (screw couplings, weld seams, moulded tanks) can lead to a sedimentation of feed materials.
- Oversized and long conveying systems, and non-continuous earthing of parts of the production plant.

¹⁷ Regulation (EC) No 1055/2006 of 12 July 2006 amending Annexes I and III to Council Regulation (EEC) N°2377/90 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, as regards flubendazole and lasalocid. OJ L 192, 13.7.2006, p. 3

- In silos or containers, differences in flow rate may cause segregation of the bulk material, which accumulates in dead zones with solidification of the bulk material.
- Conveyors which do not empty completely, such as screw conveyors and elevator boots.
- Wear of mixing equipment and conveying systems can cause a reduced flow in certain areas at which material can accumulate.
- Filter systems may accumulate residues, in particular when material featuring high dusting potential and strong aspiration flow.

The **physicochemical characteristics** of additives can contribute to cross-contamination in the following ways:

- High dusting potential, low product moisture, adherence due to electrostatic charge, as well as environmental conditions (e.g. adhesions caused by surrounding moisture) contribute to cross-contamination. The more dispersed in air and the lower the density of the components, the more sensitively they react to current fields. Basically, particle sizes < 500 µm are dispersible in the air, which facilitates the discharge of suitable, airborne components by aspiration air. An accumulation of feed material in filters and incomplete or inappropriate cleaning (see above) can lead to cross-contamination of these components into the next production batch. Also a high electrostatic loading potential, as well as higher product moistures can cause adhesions inside production plants and can result in cross-contamination.

Finally, it should also be mentioned that activities inside or outside the feed mill may contribute to undesired contamination of non-target animal feed, for instance, insufficient rinsing or no rinsing during product changes will result in a greater amount of cross-contamination. The beneficial effect of using rinsing batches can be reduced considerably if the residual material adhering to the equipment cannot be fully removed by the material flow of the rinsing batch (McEvoy *et al.*, 2003; Noser *et al.*, 2006; Dorn *et al.*, 1988). Further cross-contamination can occur at the feed plant during conveying (contaminated conveying equipment) and on-farm (e.g. during storage and transport to the feeding location).

1.3.2. Assessing cross-contamination in feed mills

In investigations involving the majority of German compound-feed plants (approximately 450), more than half of the examined production plants had a level of cross-contamination of less than 4 % (Strauch, 2002). A survey of Belgian compound-feed production companies showed similar values for pelleted products (OVOCOM, 2004, document provided to EFSA). Similar results were achieved with mashed (not pelleted) feeds (approx. 69 % containing less than 5 % cross-contamination).

These investigations, described above, refer to a general technical control of the mixing facilities used by commercial feed mills. Comparable investigations on the behaviour of coccidiostats during compound-feed production have not been carried out. As yet, analytical controls of the produced feeds for the presence of coccidiostats were only conducted in cases for which residual amounts of the coccidiostatic agents were found in food obtained from accidentally exposed animals. Systematic investigations of the behaviour of coccidiostats at compound-feed production companies have been carried out among others for lasalocid (Kennedy *et al.*, 1996, 1998; McEvoy *et al.*, 2003; Noser *et al.*, 2006). These authors concluded that:

- Cross-contamination can be reduced significantly by suitable measures.
- Contamination by coccidiostats was detected in several rinsing batches.

In a Swiss feed plant which produces feed for chickens for fattening and laying hen meal, one production passage without addition of coccidiostats was not sufficient to reduce contents of lasalocid and narasin in a laying hen meal to values below 30 µg/kg (Noser *et al.*, 2006).

More detailed investigations on the behaviour of lasalocid in the production process were carried out by Kennedy *et al.* (1996, 1998). A feed mill prepared a four ton batch of turkey grower meal containing the normal therapeutic concentration of lasalocid. The product was added in the form of a medicated premix. The first batch of non-medicated meal contained approximately 6 mg lasalocid/kg and lasalocid was still detectable in feed at concentrations in the range of 0.5 – 1 mg/kg in the ninth batch of non-medicated feed. In an experiment analogous to that described above, cross-contamination of lasalocid from a medicated premix to successive batches of non-medicated premix was measured, however, only the first batch of non-medicated premix contained any appreciable concentration of lasalocid (Kennedy *et al.*, 1996). In the same feed mill, but using a granular formulation of lasalocid, the cross-contamination from medicated to non-medicated feed decreased. Lasalocid residues, however, could still be detected in feeds up to four batches after the use of the medicated premix where lasalocid could be detected in the ninth batch (see above). Six months after the introduction of this formulation in 1995, the incidence of lasalocid residues in eggs (21 %) was lower than that found (66.5 %) in an earlier survey (1994) (Kennedy *et al.*, 1998).

1.4. Specific data for lasalocid-based feed additive products

Lasalocid sodium is stable at ambient temperatures and relatively stable to heat in neutral and acid conditions, but unstable in basic conditions as was demonstrated by stability experiments at 100°C in simple aqueous buffers. Lasalocid sodium is stable at pH 5.5 and 7.0 but unstable at pH 10.0 with a half-life of 30 min (Rose *et al.*, 1997). The compound is stable in the feed stored for three months (EFSA, 2004).

The authorised products, Avatec 15 % and Avatec 150G, are granulated containing the same lasalocid sodium homologues at the same intended concentration. In the new formulation of Avatec

150G, the organic carrier (corn cobs) is substituted by an inorganic carrier (gypsum-calcium sulphate dehydrate). Avatec 150G is described as a red-brown free flowing granular product with a mean particle size of about 500 µm. 0.1 % by weight passes a 106 µm mesh (the lowest tested). Additional information about particle size distribution, dusting potential and concentration of the additive in the dust of 15 % Avatec is not given in the Opinion of the FEEDAP Panel (EFSA, 2005). However, the low dusting potential of Avatec 150G was confirmed by a Stauber-Heubach test.

2. Methods of analysis for lasalocid

2.1. Analysis of lasalocid residues in premixes and animal feeds

In the process of approval of a feed additive containing lasalocid, the applicant presented an analytical method for quantification of lasalocid in supplemented feed. According to the current procedures, this method has been checked by the Community Reference Laboratory (EFSA, 2005). However, several other methods are in use in Member States.

The authorised level of use of lasalocid A in feed is 125 mg/kg in complete feed. For a method to be able to detect a cross-contamination, the analytical sensitivity needs to be 1.25 mg/kg complete feed which equals 1 % contamination. Multi-residue methods are not available at this level.

A validated liquid chromatography/mass spectrometry (LC-MS) electrospray method was developed to confirm lasalocid in feeds using a single quadrupole mass spectrometer. Lasalocid was extracted from the feed matrix using hexane-ethyl acetate and isolated using a silica solid-phase extraction cartridge. Lasalocid was confirmed in both medicated feeds and non-medicated feeds fortified with these drugs at the 1 - 50 mg/kg level (Turnipseed *et al.*, 2001).

Lasalocid was extracted from samples using methanol and without clean-up step derivatised with 2,4-dinitrophenylhydrazine. The derivatisation mixture was analysed directly using ultraviolet (UV) detection at 305/392 nm. The recoveries of lasalocid from spiked samples were 85 - 100 % with relative standard deviations of 4 - 10 % in a concentration range of 50 - 150 mg/kg. The limit of detection (LOD)¹⁸ (S/N=3) was 20 mg/kg (Dusi and Gamba, 1999).

An HPLC method with fluorimetric detection has been developed for the quantification of lasalocid sodium in premixes and feeds. It has been validated for repeatability and precision and tested for the

¹⁸Definitions of limit of detection (LOD), limit of quantification (LOQ), decision limit (CC α) and detection capability (CC β): Commission decision 2002/657 of 12 August 2002 implementing Directive No (EC) 96/23 concerning the performance of analytical methods and the interpretation of results (OJ L 221, 17.08.2002, p. 8-36) define the performance of analytical methods used for residue control and the interpretation of results. CC α means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant. CC β means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β . CC α is equivalent to the LOD defined by IUPAC guidance (IUPAC, 1995). The LOQ (ISO, 1997) is defined by the relative standard deviation of the estimated quantity. Generally, it corresponds to the lowest concentration tested with a relative standard deviation below the performance value needed, such as the performance for repeatability defined by Decision No (EC) 2002/657 (OJ L 221, 17.8.2002, p. 8-36).

absence of interference from the matrix (EFSA, 2004). This method has been adopted as the official Community method (Directive No (EC) 1999/76¹⁹). The LOD and the limit of quantification (LOQ) are 5 and 30 mg/kg for both premixes and feeds.

In conclusion, it can be stated that the method of Turnipseed *et al.* (2001) is sensitive enough to detect the low levels of lasalocid which might result from cross-contamination, i.e. 1 – 10 % of the prescribed level of 75 – 125 mg/kg complete feed. Hence this method should be evaluated for use in official control programs.

2.2. Analysis of residues of lasalocid in animal products

An analytical high performance liquid chromatography (HPLC) method was described and validated by the applicant for determination of lasalocid in chicken tissues (muscle, kidney, liver and skin/fat) with a LOQ of 20 µg/kg (EFSA, 2005).

According to the list of methods used by the National Reference Laboratories (NRL) for residue control, edited by the Community Reference Laboratory (CRL) (Bohm *et al.*, 2005), lasalocid residues are analysed in meat by 10 and in eggs by 6 out of 20 NRL. The Member States used different methods such as LC-DAD (LC with a diode array detector), LC-FLD (LC with fluorescence detection) and LC-MS/MS for screening and confirmatory purposes. LC-MS/MS are the most commonly used methods. The decision limits ranged between 1 and 50 µg lasalocid/kg tissue.

There is no minimum required performance level (MRPL) established for lasalocid in eggs or animal tissues.

2.2.1. Screening methods

A high performance thin-layer chromatographic (HPTLC) method was described by Bertini (2003) to screen lasalocid residue in chicken liver. The LOD was 1000 µg/kg for lasalocid.

Heller and Nochetto (2004) described a screening method based on Ion-Trap LC-MS/MS for non-polar residues in eggs. Recovery was 60 ± 20 % and the LOD was 1 µg/kg for lasalocid in eggs.

A multi-residue method based on LC-MS/MS was described by Rosen (2001) for screening of lasalocid residue in eggs and liver. Samples were extracted with methanol and purified on SPE columns before LC analysis. Screening of lasalocid was performed using multiple reaction mode transition m/z 613 > 377.

¹⁹ OJ L 207, 6.8.1999, p. 13

Dubois *et al.* (2004) described a multi-residue qualitative method based on LC-MS/MS for determining nine coccidiostats in muscle and eggs. For lasalocid residue in muscle, extraction recovery was 60 % and decision limit ($CC\alpha$) was 0.1 $\mu\text{g}/\text{kg}$.

2.2.2. Quantitative and confirmatory methods

A multi-residue method based on LC-MS was reported. The limits of detection for lasalocid were 3, 3, 7 and 3 $\mu\text{g}/\text{kg}$ in eggs, fat, liver and muscle, respectively (Hormazabal and Yndestad, 2000).

A method for the determination of coccidiostatic residues in animal tissues (chicken, pig, sheep and calf) and eggs was based on HPLC with fluorescence detection and confirmation by LC-MS/MS (Matabudul *et al.*, 2000). The LOQ for lasalocid was 1 $\mu\text{g}/\text{kg}$ in all matrices (Matabudul *et al.*, 2000, 2002).

A multi-residue quantitative method based on LC-MS/MS was described by Mortier *et al.* (2005a) for analysis of four coccidiostats in eggs. The decision limit ($CC\alpha$) was 1 $\mu\text{g}/\text{kg}$.

Rokka and Peltonen (2006) described a multi-residue method based on LC-MS/MS for the quantitative determination of four coccidiostats (lasalocid, monensin, salinomycin and narasin) in eggs and chicken meat. The decision limits ($CC\alpha$) for lasalocid were 1.4 and 1.5 $\mu\text{g}/\text{kg}$ in eggs and muscle, respectively.

A HPLC method was provided by the applicant for the quantification of lasalocid in skin/fat with a LOQ of 0.3 mg/kg.

3. Occurrence of lasalocid

3.1. Occurrence of lasalocid residues in feed materials for non-target animal species

Data on cross-contamination of feed are scarce. The Czech Republic reported the results of 254 analyses that were performed during 2006. Only 1 positive sample was found. This was a complete feedingstuff for pigs for fattening that contained 8.41 mg lasalocid/kg. The LOD of the analytical method was 0.5 mg/kg.

Denmark reported the analyses of 111 samples of feeds that were sampled between 2004 and 2007. The samples were all taken from the first batch of feed that was intended for non-target animals which was prepared following the production of feed for target animals and cleaning procedures. Only one positive sample was found containing 0.26 mg lasalocid/kg feed.

Information from the Rapid Alert System for Food and Feed (RASFF)²⁰ between April 2002 and April 2006 showed nine incidents in which lasalocid was found in feed for non-target animal

²⁰ For more information on the RASFF system: http://ec.europa.eu/food/food/rapidalert/index_en.htm

species (data provided by the European Commission). The amounts detected were between 0.003 and 12.07 mg lasalocid/kg feed and an outlier of 64.6 mg/kg feed. This outlier is most likely caused by accidental contamination.

3.2. Occurrence of lasalocid residues in animal products derived from non-target animal species

Residues of lasalocid in animal tissues and eggs can arise from cross-contamination but also if a non-target animal is given feed formulated for target animal species, intentionally or accidentally.

Eggs, muscle and liver from different food-producing animal species are analysed for residues of coccidiostats by the Member States according to requirements in Directive No (EC) 96/23. Combined results of lasalocid from 2004 and 2005 show 185 (155 in 2004 and 30 in 2005) non-compliant samples from a total of 13490 analyses. However, the results were very different in terms of detection limit and the definition of compliant and non-compliant. The levels at which a result is defined as non-compliant are not harmonised within the Member States, but several countries used 10 µg lasalocid/kg tissue as their non-compliant limit²¹. The LOD ranged between 0.03 to 100 µg/kg. Results for the analysed tissues are given in Table 2.

Table 2. Combined results of analyses of lasalocid residues in Member States in 2004 and 2005

Animal tissue	Analyses	Non-compliant
Eggs	3248	130
Poultry	4098	43
Farmed game	289	9
Pig	2350	2
Sheep/goat	1084	1
Bovine	2058	0
Rabbit	301	0
Horse	67	0

As the results from the Member States were very different in terms of the analytical methods applied, the given detection limits, and the definition of compliant and non-compliant, and the more detailed quantitative results presented by one Member State (UK) were considered in the risk

²¹ After June 2006, levels above the MRLs has been defined as non-compliant for chicken muscle, liver, skin/fat, kidney and eggs

assessment. UK has monitored residues of coccidiostats in a large number of foods over several years (UK-VMD, 1995-2005).

The results from these surveys are summarised in Table 3 below and are given in more detail in the Annex of this document. High concentrations of lasalocid were sometimes observed in chicken and quail eggs. A particularly high proportion of the quail eggs tested in targeted surveys contained lasalocid. Positive results indicating relatively high amounts of lasalocid, were also found in samples of quail muscle, chicken liver and turkey liver. No survey information was available on the amount of lasalocid residues in milk.

Table 3. Results of the UK surveys of foods for lasalocid (UK-VMD, 1995-2005).

Foodstuff	Samples tested	Samples containing lasalocid	Highest concentration detected (µg/kg)
Chicken eggs	2855	138 (4.8 %)	3450
Egg-based baby food	397	0	-
Chicken muscle	759	1 (0.13 %)	83
Fried chicken	10	0	-
Chicken-based baby food	101	0	-
Chicken liver (type of chicken not specified)	411	19 (4.6 %)	640
Broiler chicken liver	1775	7 (0.39 %)	415
Hen liver	108	0	-
Turkey muscle	72	0	-
Breaded turkey products	20	0	-
Turkey liver	341	1 (0.29 %)	120
Quail eggs	70	31 (44 %)	5400
Quail muscle	50	11 (22 %)	400
Duck liver	45	0	-
Poultry burgers	44	0	-
Rabbit muscle	112	0	-
Venison	22	0	-
Calf liver	52	0	-
Sheep liver	316	0	-
Pig liver	100	0	-
Pork sausages	100	0	-
Paté	140	0	-

Belgium has provided individual data of 958 samples of muscle tissue from different animal species and eggs that were analysed in 2005 and 2006. Five samples in eggs (26, 27, 61 µg/kg and two

without mentioned concentrations) contained concentrations above the reporting limit of 10 µg/kg (non-compliant) and nine samples were between the LOD of 2 µg/kg and the reporting limit.

In a survey of 320 egg samples, purchased in eight different European countries, eggs were analysed for the presence of nine different coccidiostats including lasalocid. Lasalocid was found in 24 samples, accounting for 17 % of all positive samples. Nearly 89 % of all the positive samples contained less than 1 µg/kg egg. Of seven samples with concentrations between 10 and 50 mg/kg, five contained lasalocid. CC α was 1 µg/kg (Mortier *et al.*, 2005b).

4. Toxicity of lasalocid

4.1. Mechanisms of toxicity

Ionophores modify the permeability of biological membranes by forming lipid soluble, dynamically reversible cation complexes and these complexes transport cations across biological membranes. Each carboxylic ionophore species has its own characteristic inorganic ion selectivity pattern. Furthermore, ionophores also differ in molecular polarity, which affects their differential distribution in biological membranes. Accordingly, there are sufficient options available among the carboxylic ionophores for each to interact with biological membrane systems in a characteristically distinct manner with consequent differences in their pathological effects.

4.2. Toxicity of lasalocid in target animal species

4.2.1. Chickens

Chickens for fattening, breeders and cage layer replacement chickens were fed diets containing lasalocid at a dose of 0, 125, 375 or 625 mg/kg feed per day, respectively, for 16 weeks (data provided by industry). Clinical pathology, haematology and histological examination at 16 weeks of age were conducted. At a concentration of 125 mg/kg feed, lasalocid did not affect the body weights, feed conversion rate, mortality or litter moisture in breeders or replacement leghorn pullets. At concentrations of or exceeding 375 mg/kg feed, lower body weights, poorer feed conversion ratios, an increase in litter moisture, and a higher mortality at days 56 and 84 of the trial were observed. Animals receiving 625 mg/kg feed showed high mortality and poor performance.

Feeding lasalocid at 125 or 375 mg/kg feed to breeder replacement pullets affected neither the fertility nor the hatchability of eggs. No signs of toxicity were observed in chickens hatched from these eggs.

In a single-dose study, lasalocid sodium was administered in the form of oral capsules to chickens for fattening, at doses ranging from 39 to 317 mg/kg b.w. Signs of toxicity occurred rapidly and included lethargy, with dropped wings and reluctance to move. Death occurred generally within 24 hours, and birds with delayed death were emaciated and dehydrated. At necropsy, nephromegaly,

splenomegaly, and hepatomegaly with scattered foci of necrosis were observed. From these data LD₅₀s of 59 and 84 mg/kg b.w. were derived. In another acute study, lasalocid sodium dissolved either in 5 % acacia gum or in 5 % of an emulsion product as the vehicle was given by gavage to chickens for fattening and oral LD₅₀s of 112 and 84 mg/kg b.w., respectively, were calculated. In a subchronic dietary study, lasalocid was administered up to a concentration of 125 mg/kg feed (equivalent to approximately 7 - 11 mg/kg b.w. per day). This concentration was well tolerated and no adverse effects were observed. Another sub-chronic study involved one day-old chickens, administered diets containing 75 to 375 mg/kg lasalocid sodium for up to 9 weeks. No significant effects on mortality, body weight, feed efficiency, or haematology were noted up to a concentration of 225 mg/kg feed. In a 13-week study with a similar design, no adverse effects up to a concentration of 150 mg/kg, were observed, but a reduction in body weight gain and feed efficiency and an increase in mortality occurred at 225 and 375 mg/kg feed.

4.2.2 Turkeys

One-day old Sun Valley SWII turkeys were allocated at random to 40 pens with either 20 males or 20 females. At 14 days of age, the number of birds in each pen was reduced to 16. Lasalocid was administered continuously from day one up to 16 weeks of age. Levels up to 375 mg/kg of feed produced no clinical adverse effects (Lodge *et al.*, 1988). In addition, no differences were observed between treated and non treated animals in the haematological and biochemical parameters examined at 12 weeks.

4.3. Toxicity of lasalocid in non-target animal species

4.3.1. Horses

A group of five horses received sequentially increasing single oral doses of lasalocid ranging between 5 and 30 mg/kg b.w, with an appropriate washout period between individual treatments (Hanson *et al.*, 1981). One of five horses died after a dosage of 15 mg/kg b.w., one of three treated horses died following the application of 21 mg/kg b.w., one of three treated horses died when receiving 22 mg/kg b.w., and one of two horses receiving 26 mg/kg b.w. did not survive. From these experiments, the author concluded that the mean lethal dose can be expected in the range between 15 – 21.5 mg/kg b.w. (Kronfeld, 2002).

Nicpon *et al.* compared the sensitivity of horses to various ionophoric polyethers, and stated that they show a higher tolerance towards lasalocid. For lasalocid, the lowest lethal doses reported was approximately 15 mg/kg, whereas salinomycin was lethal at a dose below 0.3 mg/kg (Nicpon *et al.*, 1997).

4.3.2. Sheep

A group of 17 lambs (30.9 kg) was fed lasalocid at levels up to 100 mg/kg of feed for 103 days. At these levels, lasalocid did not adversely affect growth, feed intake or feed conversion rates (Foreyt *et al.*, 1981).

4.3.3. Cattle

Groups of 6 steers were given lasalocid at a single dose of 0, 1, 10, 50 or 100 mg/kg b.w. (Galitzer *et al.*, 1986). No toxic signs developed in cattle given lasalocid at 1 or 10 mg/kg b.w. At the higher doses the earliest toxic signs were muscle tremors, tachycardia and rumen atonia. After 24 hours, animals were anorectic and had diarrhoea resulting in dehydration. At 50 and 100 mg/kg b.w. death occurred between days 1 and 22.5. Values on blood leucocytes, erythrocytes, haemoglobin, haematocrit, total protein, albumin, creatinine, urea nitrogen, total bilirubin, creatine kinase, lactate dehydrogenase, calcium, chloride and inorganic phosphate were all changed already one day after dosing. Doses as low as 5 to 8 mg/kg b.w. have been shown to cause lethal effects in young calves less than 7 days old. The clinical signs of intoxication included depression, ataxia, paresis, and paralysis with partial anorexia and alterations in the myocardium (EMEA, 2004).

Data on the effects of lasalocid in cattle submitted by applicants as part of the FDA approval for its use as a drug feed additive showed that concentrations up to 0.165 mg/kg, given for 252 days were well tolerated. Only mild and transient diarrhoea was observed at the beginning of the feeding trials in all animals given the highest dose (0.165 mg/kg), accompanied by a 30 % reduction in feed intake during the first 28 days, and a moderate decrease in feed intake during the entire study period.

In a 98 day study, lasalocid concentrations of 24, 36, 54 mg/kg dry matter were added to a 90 % concentrate barley-based diet and fed to 100 Hereford heifers to test their performance and energy partitioning. Weight gain was significantly higher in treated animals, and there were no observable effects on carcass quality. In parallel, 4 steers were fed a lasalocid diet (36 mg/kg dry matter at daily levels of 21, 44, 67 and 89 g dry matter/kg b.w.). In this experiment lasalocid was found to improve feed conversion (measured as metabolisable energy) (Delfino *et al.*, 1988).

Studies with pasture cattle given lasalocid with a corn-based concentrate indicated that up to 5 times the maximum authorised dose of 200 mg/head per day no adverse effects were observed

4.3.4. Dairy cows

Thirty-two dairy cows in mid-lactation were fed either a control (65 % alfalfa and corn silage, 35% concentrate) diet or the same diet containing 340 mg lasalocid/day for 98 days. Lasalocid did not affect milk production, or milk composition but dry matter intake was slightly lower for the animals receiving lasalocid. Lasalocid treatment improved energetic efficiency by increasing propionate and

decreasing acetate concentrations in the rumen during the first 2 weeks of treatment, but the effects became negligible by 28 days (Weiss and Amiet, 1990).

Other studies (150-200 mg/head per day) regarding the safety of lasalocid in lactating and non-lactating beef cows found that lasalocid had no adverse effect on conception or pregnancy rate i.e. no changes in gestational length and the birth weights of the calves. Effects on the cow's performance included an increase in body condition and weight, a reduction of the interval between parturition and the first post-partum oestrus (Fleck *et al.*, 1985; Goehring *et al.*, 1989; Hopman and Weber, 1986; Kiser *et al.*, 1986; Martin *et al.*, 1984; Wagner *et al.*, 1984).

4.3.5. Pigs

Sows were fed diets with lasalocid concentrations equal to intake of 130 mg/sow per day through two successive gestation periods and until parturition. The only observable effects associated with the lasalocid treatment were a higher number of live piglets at 14 days post-partum, and a lower total fat concentration in the colostrums. Body weights, rebreeding efficiency, piglet birth weight, and piglet weight at 14 days of age were comparable in all treatments (Holzgraefe *et al.*, 1986). The effect of lasalocid at a concentration equal to 140 mg/animal per day on sow reproductive performance and subsequent piglet performance during lactation was also investigated in a trial involving 114 sows. The addition of lasalocid either to gestation or lactation diets had no effect on sow's weight gains or days to return to the post-weaning oestrus. The percentages of milk protein were similar for sows in all groups, when milk samples were analysed at days 3, 7 and 14 post parturition, and there were no significant differences in litter size and piglet weights at birth, 21 days after birth, or at weaning. However, litter size and litter weight gains tended to be improved at 21 days after birth and at weaning, for sows fed lasalocid in either gestation and (or) lactation feed (Haydon and Hale, 1988).

4.3.6. Rabbits

Lasalocid sodium was considered to be toxic to rabbits with an LD₅₀ of 40 mg/kg b.w. In a developmental toxicity study with New Zealand white rabbits, lasalocid was given to groups of twelve pregnant rabbits on days 6 to 28 of gestation at dose levels of 0, 0.5, 1 and 2 mg/kg b.w. (by gavage). Treatment with lasalocid sodium was associated with a dose-related decrease in food consumption at 1 and 2 mg/kg and reduced body weight gain at 2 mg/kg, but no other signs of maternal toxicity were observed. At 2 mg/kg an increase in the number of early embryonic deaths was observed. Foetal weight was decreased in the groups given 1 and 2 mg/kg, respectively. At 1 and 2 mg/kg b.w. the incidence of foetuses with forelimb flexure was increased. At 2 mg/kg b.w. there was a slight increase in the incidence of foetuses with corneal opacity. At 2 mg/kg b.w. the incidence of foetuses with jungals connected to the maxilla was increased, as well as the number of foetuses with a complete 13th supernumerary rib and a displaced pelvic girdle, and incomplete

ossification. The NOEL for maternal as well as foetal toxicity was 0.5 mg lasalocid/kg b.w. per day (EMEA, 2004).

4.3.7. Dogs

Seventeen dogs became weak and developed neurological deficits of different degrees of severity after eating a commercial feed containing 200 mg lasalocid/kg (approximately 5 mg/kg b.w. per day) (Segev *et al.*, 2004). Twelve hours after eating the feed, the animals manifested systemic or neurological signs. Five of the dogs died, but the others improved gradually and had fully recovered by one to four days after the appearance of the clinical signs with different degrees of severity. Clinical signs consisted of quadriparesis and hyporeflexia in addition to systemic or neurological signs, including dyspnoea, a high body temperature, tongue laxity, hyperaesthesia and anisocoria. Serum biochemistry was also affected with higher activities of creatine kinase, lactate dehydrogenase and aspartate aminotransferase in the lasalocid group compared to the control group (Segev *et al.*, 2004).

In addition, there is a case report on three Spanish Bloodhound dogs (2 males and 1 female) that became ill and showed neurological signs after consumption of several fattening chickens that died on a farm nearby (Espino *et al.*, 2003). The feed was claimed to contain lasalocid at a concentration of 150 mg/kg, but this level was not confirmed by chemical analysis. On physical examination the dogs were depressed and in lateral recumbence, had tachycardia and showed mild dehydration.

4.3.8. Fish

No data were available on the effects on fish of feed containing lasalocid on fish. However the toxicity of lasalocid on aquatic organisms has been addressed by the FEEDAP Panel (EFSA, 2004). The acute toxicity of lasalocid-sodium to zebra fish *Brachydanio rerio* and *Lepomis macrochirus* was 2.5 and 3.6 mg/L (96h LC₅₀), respectively, and for goldfish 6 mg/L (72 h LC₅₀). No information on water temperature used in these studies was available.

Fish feed is unlikely to become cross-contaminated with coccidiostats since it is unlikely to be produced on the same plant as feed for target animal species, because the fat composition and the size of the granulated feed have to be changed very frequently according to the fish species and the age of the fish.

4.4. Common drug interactions

As for other ionophoric compounds, an interaction between lasalocid and pleuromutilins, such as tiamulin has been discussed. Tiamulin has been shown to be an inhibitor of cytochrome P450 isoenzymes so that co-administration with ionophoric compounds could potentially result in an

inhibition of metabolism and a decrease in the elimination of the compound as seen with monensin (Szucs *et al.*, 2004). Targeted experiments have been conducted in turkeys, as mentioned above, and showed no adverse effects during co-medication (in contrast to monensin) (EFSA, 2004; Ratz *et al.*, 1997). Moreover, data provided by the applicant to the FEEDAP panel showed that co-administration of 250 mg tiamulin/L water for three days (instead of the five day period currently recommended for tiamulin treatment of poultry) on chickens supplemented with 125 mg lasalocid per kg feed had no effect on the zootechnical performances of the animals. Considering the absence of case reports it can be assumed that the risk of drug-drug interactions with lasalocid is low. However, no data on clinical signs and other parameters such as haematology and clinical chemistry were available and these datasets were considered insufficient by the FEEDAP panel. Consequently, the FEEDAP Panel recommended avoidance of concurrent administration of lasalocid sodium with tiamulin and other drug inhibitors of the cytochrome P450 isoenzymes (EFSA, 2004).

5. Kinetics and tissue distribution

5.1. Kinetics of lasalocid in target animal species

5.1.1 Chickens

A number of kinetic studies, specifically designed to measure the organ distribution and depletion of tissue residues after cessation of treatment have been presented by the applicants, and have been reviewed by the FEEDAP Panel as well as the CVMP. Data indicate a rapid absorption with maximal plasma levels within 2 hours, and an elimination half-life of approximately 3 hours. Analysis of the radioactivity in edible tissues indicated peak concentrations of 10.3, 0.76, 1.4 and 3 mg lasalocid equivalents (total radioactive residues)/kg in liver, muscle, fat and kidney, respectively, in a study in which 16 days treatment with lasalocid (given orally in capsules) was followed by the administration of 5 mg/kg b.w. of radioactive labelled lasalocid (EMEA, 2004). The total activity recovered in the excreta represented 95.6 % of the administered dose, indicating an almost complete faecal elimination. Analysis of the excreta in a further study demonstrated that the major component was lasalocid A.

Lasalocid sodium is metabolised extensively into a number of metabolites none of which accounts for more than 10 % of the total radioactivity in the excreta, but no firm conclusion can be drawn concerning the percentage of unchanged lasalocid. The tissue residue concentrations after repeated administration of ¹⁴C-labelled lasalocid sodium (125 mg/kg for 7 days) to chickens were at 0 withdrawal time 0.29, 0.13, 0.34, and 0.05 mg lasalocid equivalents/kg in liver, kidney, skin/fat and muscle, respectively. No residue levels were found after 1 day in muscle tissue. At 5 days

withdrawal time, concentrations decreased to 0.04, 0.03, 0.04 mg equivalent lasalocid/kg in liver, kidney and skin/fat²² (EFSA, 2004).

In a published study, residual concentrations of lasalocid in fattening chickens fed medicated feed for 14 days containing 90 mg/kg lasalocid were described. The half-life of lasalocid was 11, 36 and 41 hours in serum, liver and muscle, respectively. Concentrations measured in serum at 0, 5 and 7 days withdrawal period were around 2500, 1 and 0.07 ng/mL, respectively. Lasalocid concentrations in liver were approximately 400, 20 and 10 µg/kg 0, 5 and 7 days after treatment, respectively. In muscle, initial levels after treatment (day 0) were about 10 µg/kg and levels of approximately 0.5 µg/kg remained after five and seven days (Kennedy *et al.*, 1995).

5.1.2. Turkeys

After administration for eight weeks of 125 or 200 mg lasalocid sodium/kg in feed to groups of 5 or 6 turkeys, residues of unchanged lasalocid were determined by HPLC/TLC (LOD 25 µg/kg) in muscle, liver, kidney, fat and skin after a 1, 2 and 3 day withdrawal period. Residues of lasalocid at 125 mg/kg dosage ranged from less than 25 to 85 µg/kg fresh tissue and at 200 mg/kg dosage from 25 to 2250 µg/kg fresh tissue. No residues of unchanged lasalocid were detectable in any tissue after a 3-day withdrawal period at 125 mg/kg, whereas following the administration of 200 mg/kg the skin still contained detectable levels (38 µg/kg fresh tissue). After 5 days withdrawal total labelled residues were 850 - 890 µg lasalocid equivalents/kg tissue in the liver, 120 µg/kg in the abdominal fat and 70 - 110 µg/kg tissue in the kidney, skin and fat from non-abdominal sites. Unchanged lasalocid represents only 3.8 % of the total residues in the liver, while extractable and non-extractable residues account for about 40 % and 50 %, respectively. Turkey excreta contained about 10 % of lasalocid in unchanged form, the remainder representing a large number of different metabolites (EC, 1991).

The metabolic fate of lasalocid in the turkey has been studied using ¹⁴C-lasalocid labelled in three stable positions of the carbon skeleton. A metabolic balance determination after the administration of daily doses (127 mg/kg feed) for 14 days established that after 5 days of withdrawal, 83.4 % and 80.2 % of the total administered dose had been excreted in the droppings of the males and females respectively. Biliary excretion was considerable, indicating a significant absorption and enterohepatic re-circulation of lasalocid. The metabolic fate of lasalocid was compared in chickens and rats. Methodological difficulties arose because the molecule is metabolised into a very large number of metabolites, none of which accounts for more than 1 % of the total radioactivity in the tissues or excreta. It may reasonably be concluded that lasalocid is metabolised similarly in the turkey and chicken but differently in the rat (EC, 1991).

²² Radiolabelled residues in muscle, liver, kidney and skin/fat after 5 days withdrawal period are 0, 40, 30 and 40 µg lasalocid equivalents/kg tissue, respectively, corresponding to a human daily exposure of 8 µg/kg (0.13 µg/kg b.w. per day)

5.2. Kinetics of lasalocid in non-target animal species

5.2.1. Laying hens

Five groups of layers in mid-lay were fed meals containing experimentally added lasalocid at concentrations spanning the range found as a result of unintentional contamination at a feed mill (0.1 - 5.0 mg/kg) (Kennedy *et al.*, 1996). Lasalocid was detectable in eggs within 1 day of administration of the drug. Thereafter, concentrations in eggs rose rapidly, reaching a plateau after approximately 1 week of administration. The lowest lasalocid level tested, 0.1 mg/kg feed, produced eggs containing 6 - 8 µg lasalocid/kg, whereas more than 300 µg/kg of lasalocid was detected in eggs with the highest concentration (5 mg/kg feed). Over the concentration range tested, there was a very strong correlation between the lasalocid concentration fed to the birds and the plateau concentration in the eggs. The author gave an equation of the line of best fit: concentration in eggs (µg/kg) = 63.6 x concentration in feed (mg/kg). By using this equation for 2 % (2.5 mg/kg), 5 % (6.25 mg/kg) and 10 % (12.5 mg/kg) cross-contaminated feed, concentrations in eggs of about 160, 400 and 800 µg/kg, respectively, can be expected. The correlation coefficient was 0.999. When the animals that had been fed lasalocid at a concentration of 5.0 mg/kg were transferred to a lasalocid-negative meal, lasalocid-negative eggs were produced only after the drug had been withdrawn for 10 days.

In another study, ¹⁴C-labelled lasalocid sodium was orally administered to 24 laying hens for 12 consecutive days. The product was administered daily divided in three doses in order to simulate administration with medicated feed at a concentration of 125 mg/kg of lasalocid. The eggs were collected during treatment (12 days) and after treatment for 21 days. The animals were divided into two groups of 12 animals; the first group was used to quantify the radioactive residues in albumin and egg yolk separately and the second one to study the lasalocid residues in the whole egg. The peak total radioactive residue concentrations (291 µg/kg) found in albumin were lower than those found in egg yolk (32,500 µg/kg) indicating a great affinity of the lasalocid residues to egg yolk. The concentrations of total radioactive residues in egg yolk increased during treatment until day 7 indicating that the residue steady state concentration in this tissue is very difficult to achieve. However, in albumin steady state was achieved after 3 days of treatment. The whole egg total radioactive residues followed a concentration pattern similar to egg yolk, but at lower concentrations with mean steady-state concentrations of about 11,000 to 12,000 µg lasalocid equivalents/kg. After treatment ceased the total radioactive residue concentrations declined slowly until ten days (207 µg/kg), indicating that lasalocid residues remain in the egg for a long period of time (EMEA, 2006).

5.2.2. Cattle

The metabolic fate of lasalocid sodium was studied in steers using ^{14}C -labelled lasalocid (EC, 1990). Single oral dose administration produced very low blood radioactivity levels, 89 % of the administered radioactivity being recovered in the faeces and 0.18 % in the urine after 24 hours. Some 80 % of the faecal radioactivity was extractable, divided into 54 % unchanged lasalocid and 26 % other products, representing at least 5 metabolites. None of the metabolites was present at more than 4.5 %. Lasalocid is partly absorbed and then excreted in the bile either as the parent compound or as metabolites.

Repeated administration of ^{14}C -labelled lasalocid sodium resulted in steady tissue levels, after three days with the highest residues found in the liver (EC, 1990). Of the labelled liver residues some 82 % were extractable and consisted of 15 % unchanged lasalocid, 7 % identified metabolites (5 in number) and 60 % unidentified fragments. The unidentified portion consisted of many products each representing less than 1 % of the extractable hepatic radioactivity. Some 15 % hepatic radioactivity was non-extractable. Both the extractable and non-extractable fractions were non-mutagenic when tested in the *Salmonella* reverse mutation test. The biotransformation products identified had no ionophoric properties. No further information has been provided on the identity of the various metabolites.

Weiss (1990) reported results from a study in cattle carried out in 1979. From the total distribution and elimination studies with ^{14}C -lasalocid in beef cattle, it was determined that the liver was the only edible tissue that contained substantial quantities of lasalocid-derived radioactivity at zero day withdrawal time. The levels in the liver of cattle dosed at 1 mg/kg b.w. (approximately 40 mg/kg in feed) were about 8000 μg lasalocid equivalents/kg. Using the concentration of 8000 μg lasalocid equivalents/kg in liver and linear extrapolation, cattle fed a diet cross-contaminated at a level of 2 %, 5 % and 10 % would be expected to have a total of 500, 1250 and 2500 μg lasalocid equivalents/kg, respectively.

Cattle treated with ^{14}C -labelled lasalocid sodium for 2 weeks at a rate of 1.0 mg/kg body weight (approximately 40 mg/kg feedingstuff) showed 6920, 60, 22, and 9 μg lasalocid equivalents/kg in liver, kidneys, fat and muscle, respectively, when slaughtered within 16 hours after the last dosing. After 9 days withdrawal period the total labelled residues were 713 and 19 μg lasalocid equivalents/kg in the liver and kidneys, and below the limit of detection in fat and muscle (limit of detection 3.5 $\mu\text{g}/\text{kg}$) (EC, 1990).

Cattle treated for 4 weeks under field conditions with unlabelled lasalocid sodium at a rate of 0.6 mg/kg b.w. per day (approximately 33 mg/kg feedingstuff) had liver residues of lasalocid sodium ranging from 25 - 539 $\mu\text{g}/\text{kg}$ tissue at zero withdrawal period, less than 100 $\mu\text{g}/\text{kg}$ tissue after a 2-day withdrawal period, 35 $\mu\text{g}/\text{kg}$ after a 3-day withdrawal period and no detectable residues after a 4-day withdrawal period (LOD 25 $\mu\text{g}/\text{kg}$) (EC, 1990).

Three young adult Holstein cows were orally treated with two capsules per day, at 12-hour intervals, at an average ^{14}C -radiolabelled lasalocid dose of 1.05 mg/kg per day for 14 days. The

concentration of radioactivity found during the plateau period (day 2 through 13) was an average of 44 ± 6 µg lasalocid equivalents/kg in blood and 3.2 ± 0.3 µg lasalocid equivalents/kg in milk (data provided by industry).

Cows given oral doses of 1.05 mg/kg b.w. per day of ¹⁴C-radiolabelled lasalocid in capsules (equivalent to approximately 42 mg/kg in feed) had a mean concentration of 3.2 µg/kg lasalocid equivalents in their milk. By linear extrapolation, a concentration of 0.02, 0.47 and 0.95 µg lasalocid equivalents/kg in milk would be anticipated if the cows had been given feed cross-contaminated at a level of 2, 5 and 10 %, respectively.

5.2.3. Sheep

Six male lambs (average weight 32.4 kg) were treated orally with ¹⁴C-labelled lasalocid at an average dose of 1.74 mg/kg (approximately equivalent to a dietary concentration of 44 mg/kg) per day for 14 days. The mean concentration of total radioactive residues found in blood during the treatment period was 16 ± 6 µg/L. The concentration of total radioactive residues in the edible tissues of the sheep, either at 0 or 14 day after dosing, was low, except for the liver tissue. The concentration in liver decreased from 4900 to 530 µg lasalocid equivalents/kg from 0 to 14 days withdrawal (data provided by industry). By linear extrapolation, a liver concentration of approximately 280, 700 and 1400 µg lasalocid equivalents/kg would be anticipated if the sheep had been given feed cross-contaminated at a level of 2, 5 and 10 %, respectively.

5.2.4. Pigs and fish

No studies are available.

5.2.5. Other non-target animal species

After administration of 90 mg/kg of lasalocid for 27 days to **quails**, the highest tissue concentrations were found in skin (298.3, 55, 30.8 and 33.7 µg/kg at 0, 3, 6 and 9 days after treatment, respectively) and they were ten fold higher than the muscle lasalocid concentrations (EMEA, 2004). By linear extrapolation from zero withdrawal time, a skin concentration of approximately 8, 21 and 41 µg lasalocid/kg would be anticipated if the quail had been given feed cross-contaminated at a level of 2, 5 and 10 %, respectively

After 7 day administration of medicated feed with 132 mg/kg of lasalocid sodium to **pheasants**, the liver and skin/fat lasalocid A concentrations were 28.5 and 30.7 µg/kg, respectively (EMEA, 2004).

No kinetic studies are available for other animal species.

In summary, lasalocid is absorbed by the chicken and the turkey. Elimination from tissues is slow in chicken and turkey with an elimination half-life of 11, 36 and 41 hours in serum, liver and muscle, respectively, in chickens. The liver contained the highest tissue concentrations followed by the kidney, skin/fat and muscle in both chicken and turkeys. Lasalocid is metabolised extensively into a number of metabolites, most of them hydroxylated derivatives, not exceeding 10 % of the total radioactivity in the excreta. Not all of these minor metabolites have been identified yet, but they were shown to be non-mutagenic. In pheasants, considerable concentrations of lasalocid A were found in liver and skin/fat.

Lasalocid could be detected in eggs of laying hens and in quails with a greater affinity to egg yolk. A withdrawal period of 10 days is necessary for lasalocid to be not detectable in eggs.

In cattle and sheep, the liver was the target organ for deposition of lasalocid residues and the concentration in the other edible tissues were low. In cattle liver, 82 % of the labelled residues were extractable and consisted of 15 % unchanged lasalocid.

Lasalocid could be detected in milk of cows treated with lasalocid.

6. Risk characterization

Like other ionophores, lasalocid has a narrow margin of safety between the effective dose and the toxic dose. In chickens, the main target animal species, signs of toxicity, including mortality, occur at feed concentrations that are about three times higher than the concentration authorised for the prevention of coccidiosis. In comparison with other ionophore compounds, turkeys are less sensitive to lasalocid, and co-administration of lasalocid with the pleuromutilin tiamulin did not cause signs of intoxication at the authorised dose levels to be used in the control of coccidiosis.

6.1. Animal health risks in non-target animal species associated with the accidental consumption of feed materials designated for target animal species

Dogs are particularly sensitive, showing signs of cardiotoxicity as well as neurotoxic symptoms that may be fatal, at concentrations in feed slightly above those used in poultry feeds. This sensitivity applies also to young calves.

Rabbits are also a sensitive non-target animal species. In a developmental toxicity study, the NOEL for maternal and foetal toxicity was 0.5 mg lasalocid/kg b.w. per day. Assuming a consumption of 50 g feed/kg b.w., which is applied in mono-gastric species to estimate exposure, the maximum feed concentrations authorised for chickens would result in an exposure of 6.25 mg/kg b.w., and would therefore be toxic for rabbits.

Horses, which are known to be sensitive to ionophoric coccidiostats, show a higher tolerance towards lasalocid than to other ionophoric compounds (see section 4.3.1.).

In conclusion, the available data indicate that accidental ingestion of the highest authorised level of lasalocid in poultry feed (125 mg/kg feed) for the prevention of coccidiosis may cause intoxications in non-target animal species.

6.2. Adverse health effects in non-target animal species as a consequence of cross-contamination of feed batches

The limited data available on the occurrence of lasalocid in feed materials for non-target animal species as a result of cross-contamination in feed mills show values between 0.003 and 12 mg/kg feed, corresponding to a rate of cross-contamination up to about 10 %.

In accordance with the Terms of Reference, levels of cross-contamination of 2 %, 5 % or 10 % of the highest level authorised for target animal species (corresponding to 2.5, 6.25, or 12.5 mg lasalocid per kg feed, respectively) have been evaluated. Thus at an average feed consumption of 50 g/kg b.w. in monogastric species, these concentrations would result in doses of approximately 0.1, 0.3 and 0.6 mg/kg b.w., respectively. At a rate of cross-contamination of 10 % the lowest (overall) NOEL (0.5 mg/kg b.w. in rabbits) would be slightly exceeded. The Panel concluded that adverse health effects in non-target animals are unlikely to result from cross-contamination of feed up to a level of 10 %.

6.3. Residues of lasalocid in foods derived from non-target animal species

Data on lasalocid residues in animal-derived food products are available from the monitoring system according to Directive No (EC) 96/23 and from extensive surveillance studies conducted in the UK over several years. Together with the kinetic studies available, these data indicate that the highest residue levels occur in eggs and liver tissue, whereas the residue levels in fat/skin, kidney and muscle tissue are considerably lower.

The deposition of lasalocid equivalents in eggs (particularly in the yolk) was demonstrated in connection to the extension of the MRL to eggs (EMEA, 2006). Using the Kennedy *et al.* (1996) study and the correlation between lasalocid levels in feed and eggs, the Panel estimated that concentrations from cross-contaminated feed at a level of 2 %, 5 % and 10 % (2.5, 6.25 and 12.5 mg/kg) would lead to concentrations of lasalocid in eggs of about 160, 400 and 800 µg/kg. These levels all exceed the provisional MRL for eggs of 150 µg/kg.

In surveys of foods during 11 years in the UK (see Table 3), 4.8 % of the samples of chicken eggs contained quantifiable amounts of lasalocid (between 2 and 3450 µg/kg). Considering the above kinetic calculations, it has to be assumed that the high level of 3450 µg/kg relate to the consumption of feeds not authorised for laying hens.

The high values found in some of the chicken liver samples (see Table 3) seem to be related to non-compliance with the maximum authorised dose regimens and/or the withdrawal periods, rather than reflecting levels that could result from the ingestion of cross-contaminated feed batches.

Concentrations of residues up to 5400 µg/kg of lasalocid were found in quail eggs in the UK survey. These high residue levels are most likely not associated with cross-contamination, but with a non-authorised use of feeds for laying quails. This could also explain the high level of residues that were measured in quail muscle.

In a residue study in quail (zero withdrawal period) the highest tissue concentrations of lasalocid were found in skin. Extrapolation to a level of cross-contamination of 10 % indicated a concentration in skin of about 41 µg/kg

The estimates of residues in liver (the target organ) from cattle or sheep receiving 12.5 mg/kg lasalocid sodium (corresponding to 10 % cross-contamination) was 2500 µg lasalocid equivalents/kg in cattle liver and 1400 µg lasalocid equivalents/kg in sheep liver. A level of cross-contamination of 2 % (2.5 mg/kg feed) could result in cattle liver residues of 500 µg lasalocid equivalents/kg. The monitoring of residues of lasalocid in liver samples (n = 468) in the UK surveillance study in 2005, however, did not reveal any quantifiable residues in livers of calves, pigs and sheep (see Table 3).

Extrapolation from the results of experiments with cattle indicate that a concentration of 0.02, 0.47 or 0.95 µg/kg in milk could be anticipated if the cows had been given feed cross-contaminated at a level of 2, 5 or 10 %, respectively. Surveys on residues of lasalocid in dairy milk for human consumption are not available.

6.4. Human health risk associated with residues in foods derived from non-target animal species following exposure of these animals to contaminated feed batches

The CONTAM Panel used the ADI for lasalocid of 5 µg/kg (0.005 mg/kg) b.w. established by the FEEDAP Panel to assess human health risks associated with the consumption of animal products that contained residues of lasalocid as a result of feed cross-contamination at a level of 2, 5 and 10 %. The Panel noted that the CVMP derived an ADI of 2.5 µg/kg (0.0025 mg/kg) b.w.

The values for daily human food consumption relevant for calculation of human exposure to lasalocid from cross-contaminated feed are 100 g of eggs and 100 g of liver²³.

Extrapolation of the results of residue studies suggests that such consumption of eggs from laying hens receiving feed cross-contaminated at a level of 2, 5 or 10 % with lasalocid sodium, could result

²³ Values for daily human food consumption, as defined in Directive No (EC) 2001/79²³ are for birds: 300 g muscle, 100 g liver, 10 g kidney (50 g for mammals), 90 g skin/fat in natural proportions (50 g for mammals) and 100 g eggs (and 1500 g milk). Values for mammals are given in parenthesis when they differ from bird values

in a human exposure of 0.27, 0.66 or 1.3 µg/kg b.w., respectively, corresponding to 5 %, 13 % or 27 % of the ADI of 5 µg/kg b.w.

A 60 kg consumer eating 100 g of liver from an animal fed a 10 % cross-contaminated diet could be exposed to 4.2 µg/kg b.w. from cattle liver or 2.3 µg/kg b.w. from sheep liver. The Panel noted that these exposure estimates are based on extrapolations from total radioactive residues. Limited kinetic data suggest that only a fraction of these residues (about 22 %) could be identified. This indicates that these consumption estimates can be considered to be an overestimate of the actual exposure.

In a worst case scenario, an individual could consume on the same day eggs and cattle liver coming from non-target animals fed 10 % cross-contaminated feed. In this case, human exposure could amount to 1.3 plus 4.2 µg/kg b.w., from eggs and liver respectively, i.e. 5.5 µg/kg b.w., which is slightly above the ADI of 5 µg/kg. Based on levels of lasalocid residues in liver and skin/fat resulting from authorised use in chickens for fattening, the Panel calculated that concomitant consumption of these tissues corresponds to approximately 3 % (0.13 µg/kg b.w., see 5.1.1.) of the ADI, which is considered to be negligible.

The Panel noted that the exposure estimates as calculated above are worst case estimates and do not reflect regular exposure, since exposure to residues of lasalocid resulting from cross-contamination of animal feed is likely to be infrequent.

Taking these points into account the Panel concluded that a risk of adverse health effects in consumers resulting from exposure to lasalocid residues in products from animals exposed to feed cross-contaminated even at a level of 10 %, is unlikely.

CONCLUSIONS

- Lasalocid exerts signs of toxicity typical of ionophoric compounds in various non-target animal species. Intoxications in these animal species, particularly in young animals, can be fatal, and may occur in sensitive animal species at feed concentrations close to the maximum level authorised for use in chickens and turkeys. Comparison of the available toxicological data suggests that dogs, calves, horses and rabbits are the most sensitive species.
- Adverse health effects in non-target animal species are unlikely to result from exposure to feed batches cross-contaminated up to a level of 10 % of the maximum authorised lasalocid concentration for the target animal species. At this contamination level the potential exposure of 0.6 mg/kg b.w. per day is in the region of the overall NOEL of 0.5 mg/kg b.w. per day derived from toxicological studies in rats and rabbits.
- Kinetic data and residue studies with radiolabelled lasalocid indicate that liver and eggs would contain higher concentrations of lasalocid residues than muscle tissue, skin and fat of chickens. This is supported by data from surveys performed in the UK.

- Calculations using data from experiments in chicken indicated that if laying hens are exposed to 2 % cross-contaminated feed the resulting concentration of lasalocid in eggs could be at the level of the provisional MRL for poultry eggs.
- Calculations for liver of cattle and sheep indicate that 2 to 10 % cross-contamination could result in residue levels in the range of 300 to 2500 µg/kg.
- Using the highest residue levels in eggs and liver at a level of 10 % cross-contamination and default values for daily human consumption, it was estimated that the human dietary exposure could be in the region of the ADI of 5 µg/kg b.w. as established by the FEEDAP Panel.
- Taking into account the worst case scenarios used to estimate lasalocid residues, and that consumption of eggs and liver both containing lasalocid residues resulting from 10 % cross-contamination is likely to be rare, the Panel 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 %, is unlikely.

RECOMMENDATIONS

- Feed for laying hens and other non-target animal species should be monitored frequently to prevent unauthorised use of lasalocid.
- The sensitive analytical methods that have become available for food should be validated for feed concentrations below the authorised maximum concentration to allow monitoring of the level of potential cross-contamination of feed batches during the production process.
- Analysis of milk samples for residues of lasalocid should be encouraged as no data are available at present.

DOCUMENTATION PROVIDED TO EFSA

Alpharma, 2006. Lasalocid sodium. Safety and residue data in non-target species. Confidential reports. Alpharma Animal Health Division.

IFAH (International Federation for Animal Health), 2007. IFAH-Europe report to the European Commission on The Future of Coccidiostats and Histomonostats. Final tripartite, 16 July 2007.

OVOCOM, 2004. Carry-over of coccidiostats authorized as feed additives and medicinal substances in medicated feed. Letter from the European NGOs for Agriculture (COPA-COGECA), Feed manufacturers (FEFAC) and Animal Health (IFAH) to Dr. W. Penning (Head of Unit Animal Nutrition, EU Commission, DG SANCO), dated 25 March 2004.

Belgium. AFSCA, The Food Agency.

Czech Republic. Central Institute for Supervising and Testing in Agriculture

Denmark. Danish Veterinary and Food Administration and Danish Plant Directorate.

European Commission, DG SANCO.

REFERENCES

- Bertini, S., Feirerro, S. and Berny, P. 2003. A New Improved High Performance Thin Layer Chromatography (HPTLC) Method for the Detection of Ionophore Antibiotics in Feeds and Animal Tissues. *J Liq Chromatogr Relat Technol* 26(1): 147-156.
- Bohm, D., Hahn, K. and Gowik, P. 2005. List of the screening and confirmatory analytical test methods used in the NRLs of the European Union for the substance groups A5, B2a, B2b, B2e and in the German routine field laboratories for all substance groups of Annex I, Council Directive 96/23/EC. BVL, Berlin.
- Delfino, J., Mathison, G.W. and Smith, M.W. 1988. Effect of lasalocid on feedlot performance and energy partitioning in cattle. *J Anim Sci* 66: 136-150.
- Dorn, V.P., Neudegger, J. and Knöppler, H.O. 1988. Zur Problematik der Wirkstoffverschleppung bei der Mischfuttermittelherstellung am Beispiel Nicarbazin. *Tierärztliche Umschau* 43: 524-529.
- Dubois, M., Pierret, G. and Delahaut P. 2004. Efficient and sensitive detection of residues of nine coccidiostats in egg and muscle by liquid chromatography-electrospray tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 813(1-2): 181-9.
- Dusi, G. and Gamba, V. 1999. Liquid chromatography with ultraviolet detection of lasalocid, monensin, salinomycin and narasin in poultry feeds using pre-column derivatization. *J Chromatogr A* 835: 243-246.
- EC (European Commission), 1990. Report of the Scientific Committee for Animal Nutrition on the use of lasalocid sodium in feedingstuffs for finishing cattle. Opinion expressed: 27 July 1990. ec.europa.eu/food/fs/sc/oldcomm6/antibiotics/18_en.pdf
- EC (European Commission), 1991. Report of the Scientific Committee for Animal Nutrition on the use of lasalocid sodium in feedingstuffs for turkeys. Opinion expressed: 10 July 1991. ec.europa.eu/food/fs/sc/oldcomm6/antibiotics/19_en.pdf
- EFSA (European Food Safety Authority), 2004. Update of an opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the reevaluation of coccidiostat Avatec in accordance with article 9G of Council directive 70/524/EEC, *The EFSA Journal* 77: 1-45. Adopted 1 July 2004. http://www.efsa.europa.eu/EFSA/Scientific_Opinion/opinion_feedap21_ej77_up_avatec_en1,0.pdf
- EFSA (European Food Safety Authority), 2005. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on the change of terms of the authorisation of "Avatec 15%" as a feed additive, regarding a new formulation (Avatec 150G) in accordance with Regulation (EC) No 1831/2003. *The EFSA Journal* 258: 1-8. http://www.efsa.europa.eu/EFSA/Scientific_Opinion/feedap_op_ej258_avatec_en1,0.pdf
- EMA (European Medicines Agency), 2004. Lasalocid Sodium. Summary Report of the Committee for Veterinary Medicinal Products. EMA/MRL/912/04-FINAL, October 2004. <http://www.emea.europa.eu/pdfs/vet/mrls/091204en.pdf>
- EMA (European Medicines Agency), 2006. Lasalocid Sodium (extension to eggs). Summary Report of the Committee for Medicinal Products for Veterinary Use. EMA/CVMP/46049/2006-FINAL, July 2006. <http://www.emea.europa.eu/pdfs/vet/mrls/4604906en.pdf>

- EMA (European Medicines Agency), 2007. Lasalocid Sodium. Summary Report of the Committee for Medicinal Products for Veterinary Use on the establishment of maximum residue limits. EMEA/CVMP/192132/2007, 16 May, 2007.
www.emea.europa.eu/pdfs/vet/mrls/mrlopinions/19213207en.pdf
- Espino, L., Suarez, M.L., Miño, N., Goicoa, A., Fidalgo, L.E. and Santamarina, G. 2003. Suspected lasalocid poisoning in three dogs. *Vet Human Toxicol* 45 (5): 241-242.
- Fleck, A.T., Lusby, K.S; Wagner, J.J, Garmendia, J.C and Wettemann, R.P. 1985. Protein supplementation with and without lasalocid for beef cows. *J Anim Sci* 61: (Suppl 1) 468.
- Foreyt, W.J., Parish, S.M. and Forey, K.M. 1981. Lasalocid for improved weight gains and control of *Coccidia* in lambs. *Am J Vet Res* 42 (1): 57-60.
- Galitzer S.J., Oehme, F.W., Bartley, E.E. and Dayton, A.D. 1986. Lasalocid toxicity in cattle: acute clinicopathological changes. *J Anim Sci* 62: 1308-1316.
- Goehring, T.B., Corah, L.R. and Higgins, J.J. 1989. Effects of energy and lasalocid on productivity of first-calf heifers. *J Anim Sci* 67: 1879-1888.
- Hanley, H.G., Lewis, R.M., Hartley, C.J., Franklin, D. and Schwartz, A. 1975. Effects of an inotropic agent, RO 2-2985 (X-537A), on regional blood flow and myocardial function in chronically instrumented conscious dogs and anesthetized dogs. *Circulation Res* 37: 215-225.
- Hanson, L.J., Eisenbesis, H.G. and Givens, S.V. 1981. Toxic effects of lasalocid in horses. *Am J Vet Res* 42 (3): 456-461.
- Harner, J.P., Herrman, T., Falk, J. and Behnke, K. 1996. Avoiding drug carry-over during feed processing and delivery. Kansas State University, MF-2055.
<http://www.oznet.ksu.edu/library/grsci2/mf2055.pdf>
- Haydon, K.D. and Hale, O.M. 1988. Effect of lasalocid on reproductive performance and subsequent lactation in the sow. *J Anim Sci* 66: 1877-1884.
- Heller, D.N. and Nochetto, C.B. 2004. Development of multiclass methods for drug residues in eggs: silica SPE cleanup and LC-MS/MS analysis of ionophore and macrolide residues. *J Agric Food Chem* 52(23): 6848-56.
- Holzgraefe, D.P., Jensen, A.H., Fahey, G.C., Jr. and Grummer, R.R. 1986. Effects of dietary alfalfa-orchardgrass hay and lasalocid on sow reproductive performance. *J Anim Sci* 62: 1145-1153.
- Hopman, B. and Weber, D.W. 1986. Effect of lasalocid on fall-calving beef cows. *J Anim Sci* 63: 1722-1727.
- Hormazabal, V. and Yndestad M. 2000. Determination of amprolium, ethopabate, lasalocid, monensin, narasin, and salinomycin in chicken tissues, plasma, and egg using liquid chromatography-mass spectrometry. *J Liq Chrom Rel Technol* 23(10): 1585-1598.
- ISO (International Standards Organization), 1997. 11843 Capability of detection — Part 1: Terms and definitions, Part 2: Methodology in the linear calibration case Part 2: Methodology in the linear calibration case.
- IUPAC (International Union of Pure and Applied Chemistry), 1995. Protocol for the design, conduct and interpretation of method-performance studies, *Pure Appl Chem* 67: 331.
- Kennedy, D.G., Blanchflower, W.J. and O'Dornan, B.C. 1995. Development of an ELISA for lasalocid and depletion kinetics of lasalocid residues in poultry. *Food Addit Contam* 12: 83-92.
- Kennedy, D.G., Blanchflower, W.J., Hughes, P.J. and McCaughey, W.J. 1996. The incidence and cause of lasalocid residues in eggs in Northern Ireland. *Food Addit Contam* 13: 787-794.

- Kennedy, D.G., Hughes P.J. and Blanchflower, W.J. 1998. Ionophore residues in eggs in Northern Ireland: incidences and cause. *Food Addit Contam* 15 (5): 535-541.
- Kiser, T.E., Whisnant, C.S., Rambo, T.W., Benyshek, L.L. and Peterson, L.A. 1986. The effect of lasalocid supplementation in free choice mineral on performance of beef cows, heifers, and calves. *J Anim Sci* 63 (Supl 1): 81.
- Kronfeld, D.S. 2002. Lasalocid toxicosis is inadequately quantified for horses. *Vet Human Toxicol* 44 (4): 245-247.
- Levy, J.V. and Inesi, G. 1974. Positive inotropic effect on isolated human atrial muscle produced by the ionophore X-537A. *J Clin Pharmacol* 14: 35-41.
- Lodge, N.J.A., Comben, N., Roberts, N.L. and Fairley, C. 1988. Safety of lasalocid in turkeys and its compatibility with tiamulin. *Vet Rec* 122: 576-578.
- Martin, W.M., Paterson, J.A., Smith, R.M., Morrow, R.E. and Williams, J. 1984. The effect of lasalocid supplementation in free choice mineral on performance of beef cows, heifers, and calves. *J Anim Sci* 59 (Supl 1): 108.
- Matabudul, D.K., Conway, B. and Lumley I.D. 2000. A rapid method for the determination of lasalocid in animal tissues and eggs by high performance liquid chromatography with fluorescence detection and confirmation by LC-MS-MS. *Analyst* 125(12): 2196-200.
- Matabudul, D.K., Lumley I.D. and Points J.S. 2002. The determination of 5 anticoccidial drugs (nicarbazin, lasalocid, monensin, salinomycin and narasin) in animal livers and eggs by liquid chromatography linked with tandem mass spectrometry (LC-MS-MS). *Analyst* 127(6): 760-8.
- McEvoy, J.D.G., Smyth, W.G. and Kennedy, D.G. 2003. Contamination of animal feedingstuffs with nicarbazin: investigations in a feed mill. *Food Addit Contam* 20(2): 136-140.
- Mortier, L., Daeseleire, E. and Van Peteghem, C. 2005a. Determination of the ionophoric coccidiostats narasin, monensin, lasalocid and salinomycin in eggs by liquid chromatography/tandem mass spectrometry. *Rapid Commun Mass Spectrom* 19(4): 533-9.
- Mortier, L., Huet, A.C., Charlier, C., Daeseleire, E., Delahaut, P. and Van Peteghem, C. 2005b. Incidence of residues of nine anticoccidials in eggs. *Food Addit Contam* 22(11): 1120-5.
- Murakami, K., Karaki, H., Nakagawa, H. and Urakawa, N. 1984. The inhibitory effect of X537A on vascular and intestinal smooth muscle contraction. *Naunyn Schmiedebergs Arch Pharmacol* 325: 80-84.
- Nicpon, J., Czerw, P., Harps, O. and Deegen, E. 1997. Salinomycin poisoning in a Polish stud horse. *Tierärztl Prax Ausg G Nutztiere* 25 (5): 438-441.
- Noser, J., Wenk, P., Sutter, A. and Schneider, P. 2006. Kokzidiostatika in Eiern, Geflügel-fleisch und Futtermitteln: Eine Stichproben-Kontrolle über den Nordwestschweizer Markt. *Mitt Lebensm Hyg* 97: 107-120.
- OIE (World Organisation for Animal Health), 2006. Report of the meeting of the OIE ad hoc group on antimicrobial resistance. Paris, 26–28 September 2006.
- Pento, J.T. 1986. Influence of the calcium ionophores A23187 and X537A on calcitonin secretion from the isolated perfused porcine thyroid. *Mol Cell Endocrinol* 45: 71-75.
- Pressman, B.C. and Fahim, M. 1983. Cardiovascular toxicity of ionophores used a feed additives. *Adv Exp Med Biol* 161: 543-561.

- Rätz, V., Laczay, P., Móra, Z., Csikó, G., Monostori, K., Vereczkey, L., Lehel, J. and Semjén, G. 1997. Recent studies on the effects of tiamulin and monensin on hepatic cytochrome P450 activities in chickens and turkeys. *J Vet Pharmacol Ther* 20(5): 415-8.
- Rokka, M. and Peltonen, K. 2006. Simultaneous determination of four coccidiostats in eggs and broiler meat: validation of an LC-MS/MS method. *Food Addit Contam* 23(5): 470-8.
- Rose, M.D., Rowley, L., Shearer, G. and Farrington, W.H.H. 1997. Effect of cooking on veterinary drug residues in food.6. Lasalocid. *J Agric Food Chem.* 43: 927-930.
- Rosen, J. 2001. Efficient and sensitive screening and confirmation of residues of selected polyether ionophore antibiotics in liver and eggs by liquid chromatography-electrospray tandem mass spectrometry. *Analyst* 126(11): 1990-1995.
- Safran, N., Shainberg A., Haring, R. Gurtwitz, D. and Shahar, A. 1993. Selective neurotoxicity induced by lasalocid in dissociated cerebral cultures. *Toxic In Vitro* 7: 345-352.
- Safran, N., Haring, R., Gurwitz, D., Shainberg, A., Halili, I., Levy, A., Bogin, E. and Shahar, A. 1996. Selective neurotoxicity induced by the ionophore lasalocid in rat dissociated cerebral cultures, involvement of the NMDA receptor/channel. *NeuroToxic* 17: 883-896.
- Safran N., Haring, R., Shainberg, A., Zisling, R., Futerman, A.H. and Shahar, A. 1997. Nerve cell death induced by Ca²⁺ ionophores in dissociated hippocampal cultures. Protective action of the NMDA antagonist MK-801. *Adv Exp Med Biol* 429: 207-219.
- Satoh, H., Tsuchida, K., Kaneko, K. and Otomo, S. 1992. Comparative mechanical and electrical actions of A23187 and X-537A in canine Purkinje fibers. *Gen Pharmacol* 23: 1103-1109.
- Satoh, H. and Uchida, T. 1993. Morphological and electrophysiological changes induced by calcium ionophores (A23187 and X-537A) in spontaneously beating rabbit sinoatrial node cells. *Gen Pharmacol* 24: 49-57.
- Segev, G., Baneth, G., Levitin, B., Schlosberg, A. and Aroch, I. 2004. Accidental poisoning of 17 dogs with lasalocid. *Vet Rec* 7: 174-176.
- Strauch, W. 2002. Causes and control of carry-over and cross-contamination. *Kraftfutter/Feed Magazine* 85 (4): 151-159 (Part 1). *Kraftfutter/Feed Magazine* 85 (6): 239-249 (Part 2).
- Strauch, W. 2003. Is contamination-free feed production realistic? *Feed Tech* 7 (7): 23-25.
- Szucs, G., Tamasi, V., Laczay, P. and Monostory, K. 2004. Biochemical background of toxic interaction between tiamulin and monensin. *Chem Biol Interact* 147(2): 151-61.
- Turnipseed, S.B., Roybal J.E., Pfenning, A.P., Gonzales, S.A., Hurlbut, J.A. and Madson, M.R. 2001. LC/MS confirmation of ionophores in animal feeds. *J AOAC Int* 84 (3): 640-647.
- UK-VMD (United Kingdom, Veterinary Medicines Directorate), 1995-2005. The Veterinary Medicines Directorate Annual Report on Surveillance for Veterinary Residues, New Haw, Surrey, England. <http://www.vmd.gov.uk/Publications/AnnReps/AnnReps.htm>
- Wagner, J.J., Lusby, K.S. and Rakestraw, J. 1984. Alternate supplement schedule with lasalocid for wintering range beef cows. *J Anim Sci* 1984 59, (Suppl 1) 435.
- Weiss, G. 1990. The integration of pharmacological and toxicological testing of tissue residues in the evaluation of their human food safety. *Drug Metab Rev* 22: 829-848.
- Weiss, W.P. and Amiet, B.A. 1990. Effect of lasalocid on performance of lactating dairy cows. *J Dairy Sci* 73: 153-162.

APPENDIX

Table A1. Summarised results of surveillance for veterinary residues in food in the UK (UK-VMD, 1995-2005)

Foodstuff	Samples analysed	Samples above the LOQ or reporting limit	LOQ or reporting limit (µg/kg)	Concentrations reported (µg/kg)
1995:-				
Chicken eggs	442	24	10	13 – 600
Baby food (egg based)	108	0	2	-
Chicken muscle	343	1	2	83
Chicken liver	124	2	2	49, 630
1996:-				
Chicken eggs	441	9	10	57 - 290
Baby food (egg based)	110	0	2	-
Chicken muscle	140	0	2	-
Chicken liver	187	16	2	43 – 640 (9 > MRL)
Turkey liver	6	0	Not stated	-
1997:-				
Chicken eggs	438	10	2	32 – 310
Baby food (egg based)	109	0	2	-
Chicken muscle	61	0	2	-
Chicken liver	100	1	2	120

Cross-contamination of non-target feedingstuffs by lasalocid

Foodstuff	Samples analysed	Samples above the LOQ or reporting limit	LOQ or reporting limit (µg/kg)	Concentrations reported (µg/kg)
Rabbit muscle	20	0	2	-
Turkey muscle	32	0	2	-
Venison	22	0	2	-
1998:-				
Chicken eggs	243	5	2	10 - 60
Baby food (egg based)	50	0	10	0
Broiler chicken liver	236	3	2	62 – 140 (1 > MRL)
Hens' liver	26	0	2	-
Turkey liver	58	0	2	-
Duck liver	13	0	2	-
Chicken or pig liver pâté	50	0	10	-
Poultry burgers	44	0	10	-
Rabbit muscle	50	0	10	-
Pork sausages	100	0	10	-
1999:-				
Chicken eggs	208	21	2	2 - 150
Baby food (egg based)	20	0	40	-
Baby food (chicken based)	51	0	40	-
Uncooked	34	0	40	-

Cross-contamination of non-target feedingstuffs by lasalocid

Foodstuff	Samples analysed	Samples above the LOQ or reporting limit	LOQ or reporting limit (µg/kg)	Concentrations reported (µg/kg)
chicken muscle				
Fried chicken	10	0	40	-
Broiler chicken liver	176	0	2	-
Hens' liver	24	0	2	-
Turkey liver	52	0	2	-
Breaded turkey products	20	0	40	-
Duck liver	13	0	2	-
Chicken or pig liver paté	30	0	40	-
Rabbit muscle	15	0	40	-
2000:-				
Chicken eggs	212	5	2	2 - 1400
Uncooked chicken muscle	30	0	40	-
Broiler chicken liver	197	1	2	12
Hens' liver	21	0	2	-
Turkey liver	50	0	2	-
Uncooked turkey muscle	20	0	40	-
Duck liver	12	0	2	-
Chicken or pig liver paté	30	0	40	-
Quail eggs	10	5	40	80 – 5400
Quail muscle	20	6	40	50 - 250

Cross-contamination of non-target feedingstuffs by lasalocid

Foodstuff	Samples analysed	Samples above the LOQ or reporting limit	LOQ or reporting limit (µg/kg)	Concentrations reported (µg/kg)
Rabbit muscle	15	0	10	-
2001:-				
Chicken eggs	222	0	2	-
Uncooked chicken muscle	50	0	40	-
Broiler chicken liver	210	0	2	-
Hens' liver	22	0	2	-
Turkey liver	46	0	2	-
Uncooked turkey muscle	20	0	40	-
Duck liver	7	0	2	-
Paté (type not specified)	30	0	40	-
Quail eggs	30	14	40	40 – 740 (14 > MRL)
Quail muscle	30	5	40	43 - 400
Rabbit muscle	12	0	40	-
2002:-				
Chicken eggs	280	18	Not stated	40 - 620
Uncooked chicken muscle	101	0	40	-
Baby food (chicken based)	50	0	25	-
Broiler chicken liver	312	2	Not stated	80, 100
Quail eggs	40	6	Not stated	41 - 520
2003:-				

Foodstuff	Samples analysed	Samples above the LOQ or reporting limit	LOQ or reporting limit (µg/kg)	Concentrations reported (µg/kg)
Chicken eggs	275	34	Not stated	50 - 3450
Quail eggs	30	12	40	41 - 1700
2004:-				
Chicken eggs	283	6	Not stated	38 - 200
Quail eggs	30	0	40	-
Broiler chicken liver	334	1	2 or 50	415
Hen liver	7	0	2 or 50	-
Turkey liver	66	0	2 or 50	-
2005:-				
Chicken eggs	294	1	Not stated	760
Broiler chicken liver	310	0	2 - 50	-
Hen liver	8	0	2 - 50	-
Turkey liver	63	0	2 - 50	-
Calf liver	52	0	5 - 20	-
Pig liver	100	0	5 - 20	-
Sheep liver	316	0	5 - 20	-

From 2006, MRLs for poultry muscle, skin/fat, liver and kidney, have been set at 20, 100, 100 and 50 µg/kg, respectively. Provisional MRL for poultry eggs is 150 µg/kg.

Results were taken from Annual Reports for 1995 to 2005 on Surveillance for Veterinary Residues, Veterinary Medicines Directorate (VMD), New Haw, Surrey, England; and from quarterly reports of residues surveillance that are published by VMD in MAVIS (Medicines Act Veterinary Information Service).