

**Cross-contamination of non-target feedingstuffs by monensin authorised for use as a feed additive<sup>1</sup>**

**Scientific Opinion of the Panel on Contaminants in the Food Chain**

**Question N° EFSA-Q-2005-220D**

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**PANEL MEMBERS**

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**SUMMARY**

Monensin sodium is a polyether carboxylic ionophore coccidiostat agent that is authorised as a feed additive under Commission Regulations N° 2430/1999 and 1455/2004 for use in chickens for fattening (100-125 mg/kg feed, withdrawal period 3 days), chickens reared for laying (up to 16 weeks of age; 100-120 mg/kg feed; no withdrawal period) and turkeys for fattening (up to 16 weeks of age, concentration range 60-100 mg/kg feed; withdrawal period 3 days). Despite the requirements set for feed business operators in Regulation (EC) No 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 contaminate the 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 species have been evaluated.

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Signs of intoxication in animals are consistent with the mode of action of ionophoric coccidiostats. Cardiac effects (inotropy and raised blood pressure), necrosis of striated muscles (rhabdomyolysis) and nerves (peripheral neuropathy) are reported to occur in various non-target animal species. Other signs of intoxication include anorexia, diarrhoea, depression, leg weakness, ataxia and dyspnoea as well as growth retardation. Particularly sensitive are horses, for which fatal intoxications at dosages of less than 2 mg/kg b.w. have been reported. Dogs, small ruminants and ducks are very sensitive to ionophoric polyethers. In conclusion, accidental ingestion of feed intended for turkeys or chickens containing monensin at the maximum authorised level of 120 and 125 mg/kg feed, respectively, presents a health risk for several non-target animal species.

Cross-contamination of feed at a level of 10% (12.5 mg/kg feed) of the maximum authorised monensin level for target animal species, would result in an intake for non-target animal species of up to 0.6 mg/kg b.w. per day of monensin. This level exceeds the overall no observed adverse effect level (NOAEL) of 0.3 mg/kg b.w. per day as derived from an oral toxicity study in dogs and rabbits, and hence may induce signs of intoxication in sensitive species such as horses and possibly other species. The Panel on Contaminants in the Food Chain (CONTAM Panel) concludes that adverse health effects in non-target animals may occur if cross-contamination of feed exceeds a level of 5% of the maximum authorised level of monensin in feed for target animal species.

Kinetic studies in various animal species showed that monensin is rapidly absorbed, metabolised and excreted. Kinetic data show that the highest levels of monensin residues are found in liver. Residue levels in other tissues are negligible.

Human exposure resulting from consumption of food products from non-target animal species exposed to feed cross-contaminated up to a level of 10% of the maximum authorised level, is well below the acceptable daily intake (ADI) of 3 µg/kg b.w. as established by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel). Therefore, the CONTAM Panel concluded that there is negligible risk to consumers' health from ingestion of monensin residues in tissues of animals exposed to feed cross-contaminated up to a hypothetical level of 10% of the maximum level authorised target animal species.

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

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## **BACKGROUND AS PROVIDED BY THE 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. At the switch-over from one product to the subsequent one, it is unavoidable that 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) 95/69<sup>2</sup>**

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,

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

manufacturing premixtures prepared from coccidiostats, or manufacturing compound feedingstuffs containing premixtures prepared from coccidiostats have to receive approval for 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 concerning the facilities and the equipment 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) 183/2005<sup>3</sup>**

Regulation No (EC) 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene was applicable from 1 January 2006 onwards and has replaced 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 authority 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 (EC) 70/524 concerning additives in feedingstuffs<sup>4</sup> provides that no additive may be put into circulation unless a Community authorisation has been

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<sup>3</sup> OJ L 35, 8.2.2005, p. 1

granted. This Community authorisation can only be granted if, taking into account the conditions of use, it does not adversely affect human or animal health or the environment, nor harm the consumer by impairing the characteristics of animal products.

Monensin sodium has most recently been assessed by EFSA's Panel on additives and products or substances used in animal feed (FEEDAP) (EFSA, 2004, 2007) and monensin-based products have been authorised for use as feed additive in accordance with the provisions of Council Directive 70/524/EEC (see table).

Regulation (EC) No 1831/2003 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 70/524/EEC with effect from that date.

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

<b>Coccidiostat (active substance)</b>	<b>Species or category of animals for which the use of coccidiostats is authorised (target animal species)</b>	<b>Authorised maximum content of active substance in complete feed (product name)</b>
Monensin sodium	Chickens for fattening Chickens reared for laying (max 16 weeks) Turkeys (max. 16 weeks)	125 mg/kg (Coxidin <sup>TM</sup> , Elancoban <sup>TM</sup> ) 120 mg/kg (Elancoban <sup>TM</sup> ) 100 mg/kg (Coxidin <sup>TM</sup> , Elancoban <sup>TM</sup> )

#### **4. Unavoidable cross-contamination (under practical conditions)**

Monensin sodium is authorised for use as a feed additive for the production of feedingstuffs for target animals according to the conditions of authorisation. However the production of feed containing monensin sodium can result in cross-contamination to feedingstuffs for non-target animals.

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 batches, the cross-contamination of residues is unavoidable under practical conditions.

<sup>4</sup> OJ L 270, 14.12.1970, p.1

## **5. Tolerances**

Therefore, the possibility to set tolerances for these in practice unavoidable residues of coccidiostats in feedingstuffs for non-target species should be considered in the frame of Directive (EC) No 2002/32 of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed<sup>5</sup>.

Such tolerances in feedingstuffs for non-target animals 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, levels of cross-contamination 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 animals should not have any pharmacological activity and should not threaten animal health and public health, as in some cases the tolerances for feedingstuffs for non-target animals could result in presence of residues in foodstuffs of animal origin.

### **TERMS OF REFERENCE AS PROVIDED BY THE REQUESTOR**

In accordance with Article 29 (1) (a) of Regulation (EC) No 178/2002 the Commission asks EFSA to provide an opinion of the risks involved for animal health and public health as the consequence of undesirable cross-contamination of monensin sodium authorised as feed additive into non-target feeds.

The assessment should take into account hypothetical carry over rates of 2%, 5% and 10% from feed produced with the highest authorised dose of monensin 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 species (food producing farm animals) will be assessed,
- the adverse effects as a consequence of cross-contamination of monensin sodium into feed for non-target animals,
- on the basis of the available information, an estimate of the level of residues present in food of animal origin from non-target species as the consequence of cross-contamination is performed,

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<sup>5</sup> OJ L 140, 30.5.2002, p. 10. Directive as amended by Directive (EC) No 2005/6 (OJ L 24, 27.1.2005, p. 33)

- 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 species are assessed.

## **ACKNOWLEDGEMENTS**

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## **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 (as for example chickens for fattening and chickens reared for laying) and in some cases withdrawal periods. 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 exert also 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 within a species for which the compound under consideration is authorised 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 Council Directive 70/524/EEC and Council Regulation No (EC) 1831/2003 that repeals Directive 70/524/EEC (see also the background chapter). According to these provisions, authorisation and prerequisites for use of coccidiostats 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 data provided by the applicant.

**Premixture:** A mixture of feed additives with feed materials. 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 CVMP<sup>6</sup> or the JECFA<sup>7</sup>) is used for the risk characterization and assessment. 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 factors, or the inclusion of new

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<sup>6</sup> The Committee for Medicinal Products for Veterinary Use of the European Medicines Agency

<sup>7</sup> 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).

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) 1055/2006<sup>8</sup> amending the Annexes I and III of Regulation No (EC) 2377/90<sup>9</sup> to propose maximum residue limits (MRLs) for a number of coccidiostats. The FEEDAP has also recommended MRLs for some coccidiostats, and the CONTAM Panel considered these in the evaluation process.

**Residues of coccidiostats in edible tissues, milk and eggs:** According to Directive No (EC) 96/23<sup>10</sup> 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.

**Equivalents:** Where kinetic studies have been conducted with the coccidiostat <sup>14</sup>C-radiolabelled, the concentration of total radioactive residue levels measured in the different tissues are expressed as µg parent coccidiostat equivalents/kg tissue, to indicate that these levels could be the parent compound and/or metabolites.

**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 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

Monensin belongs to the group of monocarboxylic acid polyethers produced by *Streptomyces cinnamonensis*. It exerts both, anticoccidial and antimicrobial effects. It is composed of the

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<sup>8</sup> OJ L 192, 13.7.2006, p. 3–5

<sup>9</sup> OJ L 224, 18.8.1990, p. 1–8

<sup>10</sup> OJ L 125, 23.5.1996, p. 10–32

analogues A, B, C and D with monensin A being the major component (equivalent to 98%). The chemical structure of monensin A is presented in Figure 1. In practice it is used predominantly as the sodium salt (CAS Number 22373-78-0) or as the free acid (CAS Number 17090-79-8).

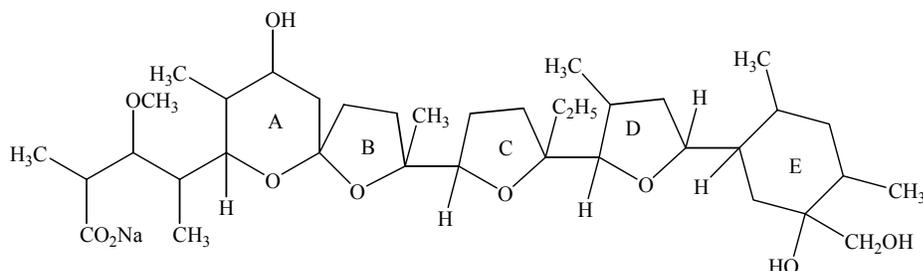


Figure 1. Chemical structure of monensin sodium A

Monensin sodium A, 2-(5-ethyltetrahydro-5-(tetrahydro-3-methyl-5-(tetrahydro-6-hydroxy-6-(hydroxymethyl)-3,5-dimethyl-2H-pyran-2-yl)-2-furyl)-2-furyl)9-hydroxy-β-methoxy-α,γ,δ,2,8-tetramethyl-1,6-dioxaspiro(4,5)decane-7-butyric acid)sodium salt, has a molecular weight of 694 and the molecular formula  $C_{36}H_{61}O_{11}Na$ . The water solubility of monensin sodium is 8.78 mg/L and the log octanol/water partition coefficient ( $K_{ow}$ ) is higher than 6.3 (EFSA, 2005).

As summarised in the background chapter, monensin sodium is authorised as a coccidiostat feed additive for chickens for fattening at a concentration range of 100-125 mg monensin sodium/kg feed, with a withdrawal period of 3 days. It is also authorised for use in chickens reared for laying (maximum age 16 weeks) at a concentration range of 100-120 mg monensin sodium/kg feed (no withdrawal period). In turkeys (maximum age 16 weeks) a concentration range of 60-100 mg monensin sodium/kg feed is authorised for the product Elancoban®, and of 90<sup>11</sup> – 100 mg monensin sodium/kg feed for the product Coxidin®, both with a withdrawal period of 3 days (Regulation (EC) No 1356/2004<sup>12</sup> and 109/2007<sup>13</sup>).

The FEEDAP has assessed the use of monensin sodium as a feed additive at up to 40 mg/kg in the diet to control coccidiosis in calves for rearing, and cattle for fattening and found that this level would be safe and effective (EFSA, 2006a).

<sup>11</sup> The minimum authorised level for Coxidin is expected to be lowered to 60 mg/kg feed based on a newly issued opinion from the FEEDAP Panel (EFSA, 2007).

<sup>12</sup> OJ L 251 27.7.2004, p.6.

<sup>13</sup> OJ L 31 6.2.2007, p.6.

## 1.1. Biological activity of monensin

Monensin is a monovalent ionophore that transports monovalent cations across biological membranes. Sodium is more efficiently transported by monensin than potassium (Gad *et al.*, 1985), and the affinity of monensin for bivalent cations is lower.

### Anticoccidial activity

Coccidial sporozoites swell up when they are exposed to monensin sodium in the intestinal lumen. Large vacuoles, pitting and holes appear in the surface of the sporozoite. This suggests that monensin causes extreme osmotic damage, which is potentially lethal to the sporozoite. Development of a sporozoite that successfully invades a host cell is inhibited as monensin continues its destructive process. Monensin selectively destroys intracellular sporozoites while remaining relatively non-injurious to the host cell. It has been shown that monensin has an effect on second-generation merozoites, but not upon developing gametocytes. Hence, the effects of monensin can be observed in sporozoites at approximately days one to five of the cycle, the first generation of merozoites on about day 16 and the second generation of merozoites at about day 19 of the life cycle (EFSA, 2006a). Hence, monensin sodium is primarily coccidiocidal and has found to be effective against coccidia that are common in avian species including *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix* and *E. tenella* of the chicken and *E. melagrimitis*, *E. pallida*, *E. dispersa* and *E. adenoides* of the turkey as well as in other protozoa (EFSA, 2005).

### Antibacterial activity

Like various other polyether ionophores, the activity of monensin against Gram-negative organisms such as *Escherichia coli*, *Salmonella spp.* and *Pseudomonas spp.* is low. Gram-positive bacteria are more susceptible to monensin, with MIC values ranging between 0.2 and 50 mg/L for *Bacteroides fragilis*, 3-50 mg/L for *Eubacterium spp.*, 3-12.5 mg/L for *Bifidobacterium spp.* and *Clostridium perfringens*, 0.1-1.6 mg/L for *Peptostreptococcus spp.*, 3-12.5 mg/L for *Lactobacillus spp.* and 1-4 mg/L for *Enterococcus faecium* (EFSA, 2004). There is no evidence to suggest that exposure of Gram-positive bacteria to monensin results in the development of cross-resistance to other antibiotics used for therapy in human and veterinary medicine (EFSA, 2004; Aarestrup, 2000; Russell and Houlihan, 2003; Mathers *et al.*, 2004). The use of monensin as a coccidiostat in chickens affected neither the colonization and shedding of *Salmonella* in the gastrointestinal tract of chickens for fattening, nor the shedding of *E. coli* 0157:H7 in cattle (EFSA, 2004).

## **1.2. Previous evaluations of the toxicological properties and the safety of monensin**

The safety of monensin has been assessed by the FEEDAP panel of EFSA as part of the authorisation process for two monensin-based feed additive products: Elancoban® and Coxidin® (EFSA, 2004; 2005; 2006a,b,c; 2007).

The FEEDAP has reviewed a large number of toxicological studies of monensin (EFSA, 2004) for Elancoban®. The studies included acute and repeat-dose toxicity studies in mice, rats, dogs (generally assumed to be very sensitive to ionophoric compounds) and rabbits. Single dose toxicity studies for a wide range of species indicated a high potential of monensin for producing acute toxic effects. Signs of toxicity were similar in all animals tested and comprised death, anorexia, hypoactivity, skeletal muscle weakness, ataxia, diarrhoea and decreased weight gain. Reported oral LD50 values (mg/kg bw) in laboratory species were as follows: rats 21.7 to 50, dogs more than 10 (female) to more than 20 (male), rabbits 41.7, mice 70 to 96, monkeys more than 60 to more than 110. Toxicological endpoints addressed included also reproductive and developmental toxicity, genotoxicity and carcinogenicity. Overall, the studies allowed the conclusion that monensin is neither genotoxic (in a bacteria/microsome reverse mutation test, an *in vitro* cytogenetics assay and an *in vivo* mouse bone marrow micronucleus test) nor carcinogenic (neither in mice nor in rats). In three-generation reproduction studies in rats, there were no adverse effects on reproduction at oral doses of up to 2.5 mg/kg b.w. per day. Maternal toxicity was seen in the rats at 3.3mg/kg b.w. per day and no developmental toxicity was seen at any dose tested (up to 3.3 mg/kg b.w. per day). Monensin was also not embryotoxic, fetotoxic or teratogenic in a rabbit developmental toxicity study at concentrations up to 0.76 mg/kg b.w.

The results of the various repeat-dose toxicity studies indicated that reduced weight gain is one of the earliest signs of intoxication. The most consistent pathological findings were degenerative changes in skeletal muscles, the diaphragm and the cardiac muscle, accompanied by an increase in the serum activity of creatine kinase and mild hepatotoxicity. In mice, a NOEL of 1.2 mg/kg b.w. per day was identified on the basis of decreased bodyweight gain and decreased leukocyte count in a two-year chronic toxicity/carcinogenicity study in male mice. In rats, the lowest NOEL was 1.4 mg/kg b.w. per day for reduced bodyweight gain in a two-year chronic toxicity/carcinogenicity study. In dogs, the lowest NOEL identified in repeat-dose toxicity studies was 2.5 mg/kg b.w. per day for various non-specific signs of toxicity in a one-year oral toxicity study. In this study electrocardiogram evaluations gave normal results in all dose groups (up to 7.5 mg/kg b.w. per day. However, haemodynamic effects were seen at lower doses in a special study of effects on cardiovascular function in dogs, in which a NOEL of 0.345 mg/kg b.w. was identified for increased blood flow through the coronary artery, whereas no adverse effects were seen at oral doses of 0.69 mg/kg b.w. or greater. The FEEDAP derived an ADI of 0 to 0.003 mg/kg b.w. per day by applying a safety factor of 100 to this NOEL for haemodynamic changes in the dog (EFSA, 2004). The

evaluation of Coxidin®, another formulation of monosodium sodium, gave generally similar results to Elancoban®. However, the lowest no observed adverse effect level (NOAEL) for Coxidin® was observed in a developmental toxicity study in rabbits at 0.3 mg/kg b.w. per day with maternal toxicity as the critical endpoint. The ADI derived from the latter study was the same as for Elancoban® applying a 100-fold uncertainty factor, i.e. 0-0.003 mg/kg b.w. per day (EFSA, 2005).

Recently provisional MRLs for chicken and turkey tissues of 25 µg/kg in skin/fat and 8 µg/kg in liver, kidney and muscle have been established by Regulation (EC) No 108/2007 and 109/2007<sup>14</sup> based on an opinion from the FEEDAP Panel (EFSA, 2006b).

Although monensin is not licensed in any veterinary medicinal products, it has now been included by Regulation No (EC) 1353/2007<sup>15</sup> in Annex I to Regulation No (EC) 2377/90 with MRLs for bovine of 30, 10, 2, 2 and 2 µg/kg for liver, fat, muscle, kidney and milk, respectively, with the marker residue being monensin A.

In the assessment of the use of monensin as veterinary medicinal product in cattle, the CVMP established a microbiological ADI of 867.7 µg per person. This level is higher than the toxicological ADI of 0-0.003 mg/kg b.w. per day, and hence in the current assessment only the toxicological ADI was considered. The CVMP proposed MRLs for bovine tissues of 30, 10, 2, 2 and 2 µg/kg for liver, fat, muscle, kidney and milk, respectively, with the marker residue being monensin A. Subsequently, by Regulation No (EC) 1353/2007<sup>16</sup> monensin was added to Annex I of Regulation No (EC) 2377/90

### **1.3. Cross-contamination of feed batches**

Feed additives, such as coccidiostats, are marketed as premixtures, 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 (see also the background chapter) 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).

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<sup>14</sup> OJ L 31 6.2.2007, p.3 and p.6.

<sup>15</sup> OJ L 303 21.11.2007, p. 6-8.

<sup>16</sup> OJ L 303 21.11.2007, p. 6-8.

Various process parameters and the 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, 1998a; Mc Evoy *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.
- 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 with 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 in 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 (Mc Evoy *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).

Systematic investigations of the behaviour of coccidiostats at compound-feed production companies have been carried out for monensin (Kennedy *et al.*, 1998a,b) and for other coccidiostats (Kennedy *et al.* 1996; Mc Evoy *et al.*, 2003; Noser *et al.*, 2006). From these investigations it can be concluded that:

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

Cross-contamination was investigated in a local poultry feed mill that was using monensin as its principal coccidiostat for chickens for fattening (Kennedy *et al.*, 1998b). Monensin, at levels in excess of 5% of the authorised dose for target animals (here approximately 110 mg/kg), was present in 22.5% of 40 samples of feed for non-target animals. Subsequent studies in the mill indicated that most of the contamination occurred during the processing of feed after the mixing stage. The mill altered its manufacturing process as a result of this study. In consequence, the incidence of feed cross-contaminated with monensin, at levels greater than 5% of the authorised dose for target animal species, fell from 22.5 to 2.5%.

Kennedy *et al.* (1998a) investigated the cross-contamination of monensin and lasalocid from medicated feed to subsequent unmedicated feeds in the feed production process. A feed mill prepared a four tonne batch of medicated meal, containing the normal therapeutic concentration of one of the test compounds. The medication was added in the form of a medicated premix.

Samples from that batch of finished feed, and from the next nine batches of finished feed, that should have contained none of the test compound, were collected. The exercise was carried out twice for each of the ionophores studied. In the result, cross-contamination of

unmedicated feeds with monensin during feed manufacture (up to eight batches of unmedicated feed contaminated) was similar to that previously observed for non-granulated lasalocid (up to nine batches contaminated).

#### **1.4. Specific data for monensin-based feed additive products**

Elancoban is a brown product that is speckled with pale straw-coloured particles. It is made from the whole dried and granulated monensin fermentation medium, mixed with a diluent (rice hulls, granular limestone) and with paraffin oil added to reduce the product's dusting potential. Two different formulations are marketed: Elancoban 200 (or Elancoban G200) and Elancoban 100 (or Elancoban G100), containing 200 and 100 g monensin per kg, respectively. Elancoban 100 and Elancoban 200 were tested for dusting potential using the Stauber-Heubach method in two studies and the particle size distribution of the dust was analysed using laser diffraction. The dust production was shown to be low with very few particles of respirable size (0.05% of Elancoban 100 and 0.1% of Elancoban 200 was of less than 9.1 µm) (EFSA, 2004). It is expected that the granulated formulations Elancoban G100 and Elancoban G200 would produce minimal dust of respirable size. Studies performed under practical conditions in packing areas indicated that 10-50% of the monensin that was dispersed in air as dust was of respirable size (<10 µm aerodynamic diameter) (EFSA, 2004).

Coxidin contains 23.7 – 26.2% monensin sodium. Sieve analysis of 10 batches showed about 70% of the additive has a particle size between 90 and 500 µm. Diffraction Laser Spectrometry (from 10 batches also) indicated (mean data) that 10% of the particles are under 19.20 (+/- 2.13) µm, 50% under 123.23 (+/- 11.20) µm and 90% of particles under 253.74 (+/- 13.33) µm (EFSA, 2005). Further information about the dusting behaviour of Coxidin is not available.

No monensin activity is lost during feed processing, including pelleting. No significant loss of monensin sodium was detected in four chicken feed batches supplemented with 100 or 121 mg/kg monensin sodium that were stored at 25°C or 37°C, with a relative humidity of 75%, for 3 months (EFSA, 2004).

## **2. Methods of analysis for monensin**

### **2.1. Analysis of monensin in premixes and animal feeds**

Monensin sodium was analysed in premix and animal feed using HPLC and post-column derivatisation with vanillin (Rodewald *et al.*, 1992). The method proved to be specific and the limit of detection (LOD) was estimated to be 0.3 mg monensin A/kg at the S/N ratio of 3. The limit of quantification (LOQ) was 5 mg/kg. An inter-laboratory study conducted in the United

States of America, Canada, France and Germany confirmed the efficacy and reliability of that method for the detection of monensin sodium in premixes and feeds and established a lower LOD (0.04 mg/kg) and LOQ (0.08 mg/kg) (Coleman *et al.*, 1997).

A LC-MS/MS method, utilising the selected reaction monitoring (SRM) mode, was used for identification and quantification of monensin in feed. Control feed samples, fortified with monensin at concentrations from 0.05 to 5 mg/kg, provided a linear response with a correlation coefficient of 0.996. The LOD of the method was 0.100 mg/kg (Ebel *et al.*, 2004).

A LC-MS electrospray confirmation method was developed and fully validated to confirm monensin in animal feeds using a single quadrupole mass spectrometer. Monensin was extracted from the feed matrix and isolated using solid-phase extraction. Monensin was confirmed in both medicated feeds and non-medicated feeds fortified at concentrations of 1 to 50 mg/kg (Turnipseed *et al.*, 2001).

Monensin in feeds was determined in an inter-laboratory study using HPLC over a concentration range of 5.5-220 mg/kg. The HPLC system used a post-column derivatisation with vanillin and UV detection. For feed samples containing monensin, repeatability standard deviation (sr) ranged from 0.9 to 7.0. Reproducibility standard deviation ranged from 1.2 to 11. Repeatability relative standard deviation (RSDr) ranged from 6.1 to 21% and reproducibility relative standard deviation (RSDR) values ranged from 8.6 to 25% (Coleman *et al.*, 1997).

*In conclusion*, several methods with the required sensitivity are available for detecting the low levels of monensin which might result from cross-contamination, i.e. 1-10% of the prescribed level of 100-125 mg/kg complete feed, and could be used for quality control purposes.

### **2.2. Analysis of monensin residues in animal products**

Analytical methods were described and validated for quantification of monensin in tissues (muscle, liver, kidney, skin/fat) of chickens and turkeys at concentrations between 6 and 90 µg/kg with a LOQ of 6 µg/kg (EFSA, 2005).

According to the list of methods used by the National Reference Laboratories (NRLs) for residue control, edited by the Community Reference Laboratory (CRL) (Bohm *et al.*, 2005), monensin residues are analysed in meat by 13 and in eggs by 16 out of 19 NRLs within the EU. The Member States used different methods such as ELISA, HPTLC, HPLC, LC/MS and LC-MSMS for screening and confirmatory purposes. LC/MS or LC/MSMS are the most commonly used methods. The decision limits ranged between 1 and 200 µg monensin/kg tissue.

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

### 2.2.1 Screening methods

Bertini (2003) described a high performance thin-layer chromatographic (HPTLC) method that could be used to screen monensin residues in chicken liver. The LOD<sup>17</sup> was 200 µg/kg.

A sensitive immunoaffinity chromatography/chemiluminescent ELISA method has been developed (Godfrey *et al.*, 1997) with a LOD around 1 µg/kg in the fat, liver, kidney and muscle tissue, but 2 µg/kg in the skin.

A multi-residue method based on LC-MS has been reported. The LODs for monensin were 1, 1, 2 and 1 µg/kg in eggs, fat, liver and muscle, respectively (Hormazabal and Yndestad, 2000).

Heller and Nochetto (2004) described a screening method based on ion-trap LC-MS/MS for nonpolar residues in eggs. The LOD was 1 µg/kg for monensin in eggs (with an LOQ of 10 µg/kg).

Dubois *et al.* (2004) described a multi-residue qualitative method based on LC-MS/MS for detection of nine coccidiostats in muscle and eggs. For monensin residue in muscle, extraction recovery was 56% and CC $\alpha$  was 0.2 µg/kg. This method is also applicable to eggs.

### 2.2.2 Quantitative and confirmatory methods

A HPLC method that has been used initially for analysis of feed was applied to the quantification of monensin in poultry tissues by Rodewald *et al.* (1992). The LOQ was 25 µg/kg for the muscle, liver and skin/fat while the LOD was 5 µg/kg.

A multi-residue LC-MS/MS method was developed to quantify monensin residues in sheep and chicken liver and chicken eggs at the LOQ level of 1 µg/kg in all matrices (Matabudul *et al.*, 2002).

A multi-residue quantitative method based on LC-MS/MS was developed to analyse four coccidiostats in chicken eggs. The CC $\alpha$  was 1 µg/kg (Mortier *et al.*, 2005a).

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<sup>17</sup> Definitions of limit of detection (LOD), limit of quantification (LOQ), decision limit (CC $\alpha$ ) and detection capability (CC $\beta$ ): 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 $\alpha$  means the limit at and above which it can be concluded with an error probability of  $\alpha$  that a sample is non-compliant. CC $\beta$  means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of  $\beta$ . CC $\alpha$  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).

Rokka and Peltonen (2006) described a multi-residue method based on LC-MS/MS for the quantitative detection of four coccidiostats in eggs and chicken meat. The CC $\alpha$  for monensin was 1.2 and 2.5  $\mu\text{g}/\text{kg}$  in eggs and muscle, respectively.

At the moment the CRL network is developing new methods and protocols.

### **3. Occurrence of monensin**

#### **3.1. Occurrence of monensin 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 premixture for pigs that contained 0.5 mg monensin/kg. The LOQ of the analytical method was 0.2 mg/kg. The concentration of monensin in the final complete feed made from this premixture would be below the LODs of the currently available methods (data provided to EFSA).

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 and prepared following the production of feed for target animals and a cleaning procedure. Two positive samples containing 0.034 and 2.46 mg monensin/kg feed were found (data provided to EFSA).

Information from the Rapid Alert System for Food and Feed (RASFF)<sup>18</sup> that was collected between April 2002 and April 2006 showed two incidents in which monensin was found in mineral feed for dairy cattle. The amounts detected were 3.19 and 11.4 mg/kg (data provided by the European Commission).

#### **3.2. Occurrence of monensin residues in animal products derived from non-target animal species**

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

Eggs, muscle and liver from different animal species are analysed by the Member States according to requirements in Directive No (EC) 96/23<sup>19</sup>. However, the results from the

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<sup>18</sup> For more information on the RASFF system: [http://ec.europa.eu/food/food/rapidalert/index\\_en.htm](http://ec.europa.eu/food/food/rapidalert/index_en.htm)

<sup>19</sup> Directive No (EC) 96/23 of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products. OL L 125, 23.5.1996, p 10-32.

Member States were very different in terms of LODs 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 use 10 µg monensin/kg tissue as their non-compliant limits<sup>20</sup>.

For monensin, combined results of 2004 and 2005 show that out of a total of 14120 samples of different animal tissues, only three samples of poultry liver were found to be non-compliant. It is not known if the non-compliant samples are from target or non-target animals. The LOD ranged from 1 to 50 µg/kg. No details are given of the concentrations of monensin residues that were found in the three non-compliant samples.

Belgium has provided individual data for 958 samples of muscle tissue from different animals and eggs that were analysed in 2005 and 2006. Four samples from chicken, one sample each from egg, sheep muscle, duck muscle and poultry muscle, contained concentrations of monensin that were greater than the LOD of 2 µg/kg but less than the Belgian non-compliant limit of 10 µg/kg. None of the samples contained residues of monensin that were greater than 10 µg/kg (data provided to EFSA).

Monensin has been analysed in United Kingdom surveys for residues of veterinary drugs in food and reported over an eleven-year period from 1995 to 2005 (UK-VMD, 1995-2005). These results showed a few samples of liver containing residues of monensin. Seventeen samples of chicken liver out of 2205 samples collected (0.8%) had measurable residues of monensin of up to 25 µg/kg; 3 out of 3077 sheep liver samples (0.1%) had residues of up to 56 µg/kg; and 2 samples out of 292 cattle liver samples (0.7%) had residues of up to 11 µg/kg. The other samples that were analysed were all negative for the presence of monensin (reporting limits = 2 to 5 µg/kg): chicken eggs (2118 samples), raw chicken muscle (530), raw turkey muscle (140), turkey liver (382), quail muscle (41), pheasant (2), rabbit (116), venison (20), pig liver (128), pâté from chicken or pig liver (101), chicken-based baby foods (100), egg-based baby foods (50), canned poultry (40) and breaded turkey products (20). No data were available for monensin residues in milk.

In a survey of 320 egg samples, purchased in eight different European countries, eggs were analysed for the presence of nine different coccidiostats including monensin. Monensin was found in three samples with concentrations around 0.3 µg/kg (Mortier *et al.*, 2005b). In this article previous findings from the laboratory were also described: 190 egg samples were analysed for monensin in 2004 and one positive sample was found with a concentration of 10 µg/kg. In another survey in Northern Ireland, 161 eggs were analysed in 1994. Six of them contained monensin at a concentration below 2.5 µg/kg (Kennedy *et al.*, 1996).

It is not possible to draw from any of these survey results any meaningful conclusion about the incidence of residues in foods, as the foodstuffs were not selected randomly. However, the

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<sup>20</sup>After February 2007, levels above the provisional MRLs are non-compliant for chicken and turkey muscle, liver, kidney (8 µg/kg) and skin/fat (25 µg/kg).

highest concentrations of residues detected may give an indication of the maximum levels of monensin contamination of foods that could be expected under practical conditions.

#### **4. Toxicity of monensin**

##### **4.1. Mechanisms of toxicity**

Ionophores modify the permeability of biological membranes by forming lipid soluble, dynamically reversible cation complexes. These complexes facilitate the transport of 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.

Monensin has a small therapeutic margin. Signs of intoxication, related to the secondary pharmacological effects including primarily cardiovascular effect (ionotropic activity due to changes in cation transport) and effects on skeletal muscles of monensin are very similar in all animal species, whereas the susceptibility shows marked species differences. These species differences seem to be related to the rate of metabolism and magnitude of a first-pass effect in individual species, the lowest overall rate of biotransformation being observed in horses, known to be one of the most sensitive animal species.

##### **4.2. Toxicity of monensin in target animal species**

###### **4.2.1. Chickens**

The toxicity of monensin for chickens for fattening has been reviewed and summarised by the FEEDAP Panel. The tolerance studies submitted by the applicant suggested that a level of 125 mg monensin/kg complete feed was safe when fed to chickens for fattening and chickens reared for laying. At this dose level, with the exception of a slightly lower body weight gain (females only, and associated with a slightly lower food and concurrent lower water consumption), there were no notable clinical, laboratory or pathological findings. By contrast, a moderate to marked dose-related lower body weight gain was observed at 250 mg monensin/kg feed (EFSA, 2004, 2005).

###### **4.2.2. Turkeys**

The toxicity of monensin for turkeys for fattening has been reviewed and summarised by the FEEDAP Panel. The tolerance studies submitted by the applicant suggested that levels of 100 mg monensin/kg complete feed was safe when fed to turkeys for fattening. With the exception of a slightly lower body weight gain in females, which was associated with slightly lower food consumption and concurrent slightly lower water consumption (both also seen in males), there were no notable clinical, laboratory or pathological findings at this dose level. No details

of the parameters were given. In contrast, all the animals receiving 200 or 300 mg/kg feed showed a slight/moderate dose-related reduction in body weight gain coupled with slightly lower food and water consumption and/or slightly reduced blood phosphorus and triglyceride levels (EFSA, 2004, 2005). Bodyweights were significantly depressed by 150 mg/kg of monensin in the fifth week after hatching, but there was no significant depression in feed consumption (Czarnecki, 1990).

For both turkey and chicken, the FEEDAP Panel noted the small margin of safety (of less than twice the maximum authorised dose) which is common for ionophores. The margin of safety could not be determined more precisely due to large dose intervals in the corresponding experiments.

### **4.3. Toxicity of monensin in non-target animal species**

#### **4.3.1. Laying hens**

Laying hens are considered to be the animals most likely to be in contact with monensin-containing feeds, as one of the commercial products is authorised for use in chickens reared for laying until the age of 16 weeks (112 days). To demonstrate the safety in laying hens, groups of 6 Heisdorf and Nelson laying hens, 316 days of age were fed diets containing monensin at concentrations of 0, 88, 264, or 440 mg/kg for 2 weeks, except the 88 mg/kg group which continued treatment for another 4 weeks (data provided by industry). On day 15 of the test, the diet of the group that had received 264 mg/kg was changed to a ration containing 88 mg/kg. Also, on day 15, the group that had previously been fed a diet containing monensin at 440 mg/kg had its ration changed to a basal diet that contained no monensin. All hens on 264 and 440 mg/kg monensin groups ceased egg production during the first 14 days. At 440 mg/kg, hens completely ceased egg production after 7 days of treatment. Feed intake was greatly depressed in both the 264 and 440 mg/kg monensin groups. Neither feed intake nor egg production were affected in the group that was fed diet containing 88 mg/kg for 6 weeks.

#### **4.3.2. Ducks and guinea fowl**

Industry provided data on effect in 10-days old Mallard ducklings when fed diets containing monensin at concentrations of 0, 62, 160, 365, 900, 2250 or 5000 mg/kg for 8 days. At dietary concentrations of 160 mg/kg or higher mean body weight gain values for birds fed monensin sodium were significantly lower ( $P \geq 0.05$ ) than in the control animals. Food consumption for groups that received monensin sodium at concentrations of  $\geq 900$  mg/kg was also lower than for the controls. The no-observed-effect level for this study was a 62 mg monensin sodium kg feed.

In guinea fowls, the LD<sub>50</sub> value for monensin was 95 mg/kg b.w. (Kamphues *et al.*, 1990), which is lower than that measured for chickens (200 – 231 mg/kg b.w.; EFSA, 2004).

#### **4.3.3. Ostriches**

Monensin was accidentally fed to 104 farmed ostriches at a concentration of 215-224 mg/kg for 13 days (Baird *et al.*, 1997). Signs of toxicity were observed in a total of 42 birds. Initial clinical signs were muscle weakness and ataxia, which progressed to recumbency, dyspnoea and death, despite intensive supportive therapy. Serum activities of creatine kinase, aspartate aminotransferase and lactate dehydrogenase were high in the intoxicated birds. Postmortem analysis showed few gross lesions. Widespread degenerative myopathy of skeletal muscle was seen, but there was no histological evidence of cardiomyopathy in any of the birds examined. Finally, such clinical cases did not occur after the withdrawal of the monensin-contaminated feed, but all the birds showing clinical signs of toxicity died or were euthanised (Baird *et al.*, 1997).

#### **4.3.4. Horses**

Horses are generally considered to be very susceptible to monensin. It has been described that the consumption of cattle feed (33 mg/kg) cause transient anorexia, whereas the consumption of 121 mg/kg (feed for chickens for fattening) causes clinical signs of toxicity ultimately resulting in death. However, the actual amount of feed ingested, and hence the effective dose was not reported in these cases (Matsuoka, 1996).

The clinical signs of monensin toxicity in horses include partial to complete anorexia, colic pain, sweating, tachycardia, uneasiness, polyuria, progressive ataxia, recumbence and death. Post mortem investigations reveal haemorrhage and pale areas in the heart (in contrast to pigs and dogs in which the lesions are most prominent in skeletal muscle tissue), and in some cases pulmonary edema, hydrothorax, ascitis and inflammatory reactions in the stomach and the intestines (Novilla and Folkerts, 1986). Animals that die soon after exposure, often do not show these typical lesions, since they had not had the time to develop these. Evidence of degenerative cardiomyopathy and congestive heart failure was found following histopathological examination, confirming that in horses the heart is the primary predilection site of monensin toxicity. Amend *et al.* (1980) describes different stages of monensin toxicity in horses: high toxic doses given experimentally by gavage may induce acute death in less than 24 hours. Exposure to non-lethal concentration results in slower onset of toxicity with lethality occurring delayed (up to 14 days). This delayed response is caused by progressive congestive heart failure developed by the animals.

In one of the few controlled experiments, monensin was given once to 2 horses by gavage at dosages of 1, 2, and 3 mg/kg b.w. (Hanson *et al.*, 1981). One of the horses died after a dosage of 2 mg/kg b.w. and the other horse died after a dosage of 3 mg/kg b.w. The clinical signs of toxicosis observed in horses were progressive, and included depression, ataxia, paresis, and paralysis with partial anorexia. Intermittent profuse sweating was observed before death in horses given monensin. From this study a LD<sub>50</sub> of 1.38 mg/kg b.w. was estimated. The findings differ from field observations, in which horses consumed obviously smaller amounts of contaminated feed only. Moreover, in the light of the small number of observations this level can only be considered as an indication.

One of the likely reasons of the high susceptibility of horses to monensin is their relative deficiency in CYP450 expressing demethylating enzymes and hence the slow clearance of monensin. In vitro experiments with liver microsomes from various animal species, including horses, pigs, broiler chickens, cattle and rats, showed that horses has the lowest catalytic efficiency to demethylate (and hence detoxify) monensin (Nebbia *et al.*, 2001). These findings seem to explain also some of the inconsistencies in the outcome of intoxications under field conditions.

#### **4.3.5. Cattle**

The FEEDAP Panel has assessed monensin sodium for use as a feed additive in young cattle to control coccidiosis (EFSA, 2006a). It was concluded that tolerance tests indicated that a dose range of 30 to 40 mg/kg complete feed can be considered safe for calves for rearing and cattle for fattening. No signs of toxicity (as detectable by haematology, blood biochemistry and necropsy) were observed at dietary concentrations of up to 110 mg/kg for beef cattle and 120 mg/kg for calves. These finding suggest a margin of safety of approximately 2.5. The FEEDAP Panel commented that the margin of safety for accidental overdosing (no longer than 10 days) would probably be considerably higher than 2.5, because the high doses of monensin sodium would cause a rapid and drastic depression in feed consumption (EFSA, 2006a).

The toxicity for dairy cows is not expected to be significantly different from those of young cattle for rearing.

#### **4.3.6. Water buffaloes**

The consumption of monensin-containing feed from a feedlot in which cattle and buffaloes were kept together resulted in deaths of water buffaloes. The susceptibility to monensin toxicity was compared between 3 bovine calves and 3 buffalo calves orally dosed 5, 7.5 or 10 mg monensin /kg b.w.. Only the buffaloes became ill and died with clinical signs initiating at 18-20 hours post dosing. The clinical signs were comparable to a field case where anorexia, muscular weakness, dyspnea and recumbency. Segmental necrosis of myofibres were

observed but histopathological changes were more pronounced in the myocardial cells. These field and experimental cases suggest that buffaloes have a lower tolerance to monensin than cattle (Rozza *et al.*, 2006).

#### **4.3.7. Sheep**

Intoxications by monensin at doses of 40-50 mg/kg in feed for a few days have been reported in sheep (Sályi *et al.*, 1988). The clinical symptoms were prostration, incoordinated movements, weakness of the posterior body half and dyspnoea. The pathological examination revealed extended Zenker's degeneration in the skeletal musculature (mostly in the large femoral muscles) showing a similar (uniformly acute or restorative) stage, characterised by the primary damage of the outer membrane and structure of myofibrils, as well as of certain cell organelles (mainly mitochondria). In the myocardium fatty infiltration and mild swelling of the mitochondria were found.

Groups of 10 lambs of each sex received monensin at a dose of 0, 20, 60 and 100 mg/kg feed for 3 months (data provided by industry). One lamb in the 100 mg/kg treated group died on day 9. All other lambs survived. There was a treatment-related decreased average daily weight gain and feed efficiency at dose levels of 60 and 100 mg/kg. Also, there were statistically significant changes in kidney (60 and 100 mg/kg) and heart weights (100 mg/kg). No pathological changes were seen in the kidneys or urinary bladders. Gross examination revealed moderate skeletal muscle degeneration. Monensin related lesions in the cardiac muscle were found which consisted of focal degeneration at 100 mg/kg (two animals) and at 60 mg/kg (one animal). It can be concluded that monensin at doses of 60 and 100 were toxic to the lambs.

In male lambs for fattening (aged 3-4 months and weighing 16-28.5 kg) a single oral dose of 5 mg/kg b.w. of monensin caused a temporary loss of appetite but no changes in behaviour or the general state of the animals were observed (Donev *et al.*, 1980). The animals died 72 to 120 hours after doses of 10 and 30 mg/kg b.w.. The clinical signs of toxicity were characterized by anorexia, ataxia, paresis, paralysis of the limbs and tachycardia. Following 30-days administration of monensin at doses of 10 and 50 mg/kg in feed, no negative effects on the behaviour, general condition, or blood chemistry were seen.

Monensin toxicosis was induced in lambs by either a single oral dose of 12 mg/kg b.w. or six daily doses of 8 mg/kg b.w. (Confer *et al.*, 1993). Clinical signs of toxicosis consisted of depression, dyspnoea, stiffness of gait, reluctance to move and recumbency. Increased serum creatine phosphokinase activity was observed and histopathological examination revealed segmental necrosis in cardiac and skeletal muscle. Muscle fibre necrosis was more severe in skeletal than cardiac muscle and most severe in sheep given monensin daily at a dose of 8 mg/kg.

Monensin was administered orally to sheep at doses of 12 (the LD<sub>50</sub>), 16, and 24 mg/kg b.w. (Anderson. *et al.*, 1984). Clinical signs of monensin toxicosis were observed within 24 to 36 hours of administration. Clinical signs included CNS depression, anorexia, diarrhoea, and stiffness. Serum creatine phosphokinase and aspartate aminotransferase activities were increased. At necropsy there were skeletal muscle haemorrhages, pale myocardium, and pulmonary oedema but no microscopic lesions were observed

#### **4.3.8. Goats**

Only one report has been published involving goats that were given monensin. At a concentration of 55 mg/kg in feed for 3 weeks anorexia and diarrhoea, along with increased pentobarbital sleeping time and serum lactate dehydrogenase level were seen. Goats fed 8 mg/kg or 11 mg/kg in the feed for 5 days showed no signs of hepatotoxicity (Dalvi and Sawant, 1990).

#### **4.3.9. Pigs**

In pigs, anorexia, diarrhoea, lethargy, dyspnoea, ataxia, knuckling at the fetlock and myoglobinuria progressing into lateral recumbency and death have been described in cases of acute intoxication, but in many case reports the actual dose was not mentioned (cited from Novilla, 2007). Dilov *et al.* (1981) conducted the first studies on the tolerance of pigs towards monensin. In this study, a total of 46 pigs weighing 15 to 60 kg received Elancoban 100 (monensin 10 %) by a single gavage dose or in the feed. The gavage dose of 5 mg monensin per kg b.w. did not lead to overt signs of toxicity. Gavage doses of 10 and 20 mg/kg monensin produced toxic effects, and 30 mg/kg was lethal (Dilov, 1981). In controlled feeding experiments, Van Vleet and Ferrans (1984) gave monensin to pigs at a dose of 40 mg monensin/kg b.w.. Animals were killed after 1, 2, 4 and 16 days (2 animals per interval) of exposure, respectively. Already on the first day, extensive cardiac necrosis with contraction bands were present. Necrosis of cardiac myocytes and phagocyte invasion into the necrotic areas could be seen on days 2 and 4. Surviving myocytes were condensed and showed marked myofibrillar lysis and sarcoplasmic vacuolization (days 2,4 and 6). The authors concluded that monensin cardiotoxicity in pigs constitutes a unique sample of selective injury to the atrial myometrium.

Groups of ten growing finishing pigs of each sex were fed monensin at a concentration of 0, 50, 150 or 500 mg/kg of feed for 111 days (data provided by industry). Four of the pigs that received 500 mg/kg died (sex not stated). Two other pigs from the same group showed signs of toxicity on day 1 (recumbent, cyanosis) and the other two pigs showed signs of degenerative myopathy. One also had a chronic severe nephrosis. Growth was slightly depressed in the surviving pigs in the 500 mg/kg group. No changes in haematology, urinalysis and blood chemistry were seen other than elevated serum aspartate

aminotransferase activity. The values for the 500 mg/kg treatment were higher than the other treatments at all post-treatment sampling times. It can be concluded that monensin sodium at a dose of 500 mg/kg in feed is toxic to pigs and therefore a NOAEL of 150 mg/kg in feed was identified.

Monensin was fed to 18 gilts at doses of 0 or 100 mg/kg (data provided by industry) throughout the growing, finishing, breeding, gestation and lactating stages. There were no treatment-related effects on reproductive performance compared with the control group, but the detailed descriptions of effects were not available.

Monensin was fed to 8 gilts and 6 sows at doses of 0 or 100 mg/kg from 10 days prior to breeding, continuing through 114 days of gestation and 28 days of lactation (data provided by industry). A similar control group was fed rations not containing monensin. There were no treatment-related effects on conception rate, litter size at birth, survival of pigs or growth rate of offspring to weaning.

#### **4.3.10. Rabbits**

From acute toxicity experiments an oral LD<sub>50</sub> was calculated to be 41.73 mg monensin/kg b.w. (data provided by industry).

A developmental toxicity study was performed on pregnant Dutch belted rabbits that were given oral gavage doses of 0, 0.076, 0.38 or 0.76 mg/kg b.w. per day on days 6-18 of gestation (EFSA, 2004). The control group consisted of 25 animals and the treatment groups each consisted of 15 animals. A dose-dependent decrease in food intake was seen in the does but maternal bodyweight gain was unaffected. No adverse pathology was seen at *post-mortem* of the does. There was no effect on the number, uterine location, sex or weight of the foetuses and there were no effects on the numbers of *corpora lutea* and resorptions. The incidence of external, skeletal and soft tissue abnormalities of the fetuses was not affected by treatment. It was concluded that monensin was not embryotoxic, foetotoxic or teratogenic in the rabbit at oral doses of up to 0.76 mg/kg b.w. per day.

#### **4.3.11. Fish**

The FEEDAP Panel has evaluated the toxicity of monensin sodium on aquatic organisms (EFSA, 2005). The acute toxicity of monensin sodium was tested in rainbow trout (*Oncorhynchus mykiss*) and a 96-hr LC<sub>50</sub> value of 1.88 mg/L was calculated

Juvenile rainbow trout were exposed for 96 hours to a range of concentrations of monensin sodium, the NOEC and the acute toxicity (96-hr LC<sub>50</sub>) values were 0.70 mg/L and 9.0 mg/L

respectively. In bluegill (*Lepomis macrochirus*) a NOEC of 3.1 mg/L and the 96-hours LC<sub>50</sub> was 16.6 mg/L.

## **5. Kinetics and tissue distribution**

The kinetics of monensin following oral administration has been evaluated in chickens, turkeys and cattle (and rats). Several studies with both <sup>14</sup>C-labelled monensin and unlabelled compound have shown that, following oral administration, monensin is rapidly absorbed and extensively metabolised by the liver and that most of the administered monensin and its metabolites are excreted in the bile. The metabolite pattern for monensin is similar in cattle, chickens, rats and turkeys. Metabolite characterization studies indicated that decarboxylation, hydroxylation, o-demethylation and oxidation are the primary metabolic pathways for monensin resulting in low concentrations (less than 10% of the total radioactivity) of a large number of polar metabolites in the faeces of monensin-dosed animals (EFSA, 2004, 2005, 2006c; EMEA, 2007).

### **5.1. Kinetics of monensin in target animal species**

The kinetics of monensin in chickens and turkeys has been reviewed extensively recently by the FEEDAP Panel (EFSA, 2004, 2005, 2006c).

#### **5.1.1. Chickens**

<sup>14</sup>C-labelled monensin sodium was administered to male and female chickens in the feed for eight consecutive days at a concentration of 125 mg/kg. Animals were killed 0, 1, 2 or 3 days after withdrawal of supplemented feed. At 0, 1 and 2 days withdrawal the liver had the highest concentrations of <sup>14</sup>C-radiolabel, with a rapid exponential decline ( $t_{1/2} = 0.9$  day). A much slower exponential decline was observed ( $t_{1/2} = 3$  days) in the skin/fat which had the highest residue concentrations after the three-day withdrawal. At 0-day withdrawal, the total radioactive residues, expressed as monensin equivalents, was 1660, 360, 340 and 90 µg/kg in liver, skin/fat, kidney and muscle, respectively. At 3-days withdrawal, 160, 210, 80 and 40 µg monensin equivalents/kg was found in liver, skin/fat, kidney and muscle, respectively. The parent compound, monensin sodium, was present at a low level in the liver (20 µg/kg) and skin/fat (10 µg/kg) at 0-day withdrawal. Concentrations of parent monensin were below the LOQ (6 µg/kg) in the kidney and muscle at 0-day and in all relevant tissues at one-day and subsequent withdrawal times (EFSA, 2006c).

Studies with <sup>14</sup>C-labelled monensin in the chicken, steer, calf and rat have been published (Donoho *et al.*, 1978; Donoho *et al.*, 1982; Davison, 1984). Data indicate that monensin sodium is absorbed to a limited extent (11-31%) and eliminated rapidly mainly through the faeces (99% after 48 hours) as parent monensin and metabolites, biliary excretion

representing about 10-15%. Monensin sodium is metabolised extensively and gives rise to six metabolites which are identical to those formed in the rat and steer and correspond to the demethylation of the methoxy-group (see Figure 1) and the subsequent oxidation of the resulting hydroxy group (keto derivative), the decarboxylation of the molecule and the concomitant mono- or dihydroxylation at different but undetermined positions of the E, D, B and C rings. The same metabolites have been found in the excreta and the tissues (EFSA, 2005, 2006c).

### **5.1.2. Turkeys**

The bioavailability of monensin sodium in turkeys is low (0.8%). 65% of the radioactivity from ingested <sup>14</sup>C-labelled monensin is excreted within 24 hours and 78% after 48 hours (EFSA, 2005).

The residues of monensin sodium that were found in tissues of turkeys, following continuous administration of 100 mg monensin/kg in feed for 84 days, were 186, 8, 8 and 3 µg/kg in skin/fat, kidney, muscle and liver, respectively. Residues were not detectable after 1-day withdrawal in liver and kidney but 670 and 9 µg/kg were measured in skin/fat and muscle, respectively. Residues persisted in skin/fat and concentrations of 64 and 136 µg monensin/kg were found after 2 and 4 days, respectively (EFSA, 2005)

## **5.2. Kinetics of monensin in non-target animal species**

### **5.2.1. Cattle**

The metabolic fate of monensin sodium has been evaluated in bovine (EFSA, 2006b, EMEA, 2007). In cattle, 75% of the radioactivity ingested was recovered in the faeces after three days, and nearly all of the administered material was recovered after 7-11 days. Only very limited urinary excretion (1–3%) was observed. Significant biliary excretion was observed (35% of the administered dose in calves). Parent monensin represented less than 3% of the biliary radioactivity but 50% of the total radiolabel recovered in the faeces. A large number of metabolites are formed by oxidative metabolism catalysed by CYP450 enzymes (probably CYP3A), from which only six have been isolated and identified from bovine excreta and bile. total monensin metabolism was highest in cattle, intermediate in rats, chickens and pigs and lowest in horses (Nebbia, 2001). The major metabolites resulted from the demethylation, decarboxylation and/or hydroxylation of monensin sodium which each represented less than 5% of the total radioactivity. Parent monensin represented less than 10% of total radiolabelled residues in the liver and in the faeces and 2% in milk. The metabolic profile in the faeces of monensin in cattle is qualitatively similar to that in the rat (EFSA, 2006b; EMEA, 2007). The major metabolites were also evaluated in *in vitro* assays for the antimicrobial and ionophoric activity. The study of biological properties of one of the six O-demethylated metabolites

identified in bovine indicated that the metabolite exhibited only 5% of the activity of the monensin sodium. From these data EMEA concluded that in a conservative estimate, based on all available information, monensin metabolites retain no more than 50% of the pharmacological and microbiological activity of the parent compounds (EMEA, 2007).

Cattle for fattening were given  $^{14}\text{C}$  monensin sodium in their feed at a concentration of 33 mg/kg for 5 days. The amount of radiolabel in kidney, muscle and fat was less than the LOD of 25  $\mu\text{g}/\text{kg}$  after a 12-hour withdrawal. However, mean total residue levels (expressed as mg monensin equivalent/kg tissue) of 332 were found in liver (EFSA, 2006a). By linear extrapolation, a concentration of 25, 63 and 130  $\mu\text{g}$  monensin equivalents/kg liver would be anticipated if the cattle had been given feed cross-contaminated at a level of 2, 5 and 10% (2.5, 6.25 and 12.5 mg/kg), respectively.

Recently, the presence of monensin residues in milk after feeding lactating cows with monensin has been evaluated (Bagg *et al.*, 2005). The doses administered were 72, 144 and 240 mg monensin/kg in the feed for a period of 21 days. The highest dose corresponds to a dose of 4865 mg monensin/day. Milk samples were collected on days 0, 1, 2, 5, 7 and 20. There was no detectable monensin residues (LOQ 5  $\mu\text{g}/\text{L}$ ) in any of the milk samples collected. These results support previous findings on monensin residues in lactating cattle, where monensin was not detected in milk at the same LOQ. The highest level of monensin administered in these studies were 1125 and 1274 mg/day. In the Summary Report on monensin provided by EMEA, additional data are presented, confirming that parent monensin as well as the metabolite M-6 can be excreted with milk. Under steady state conditions following repetitive treatment for 9 days by intraruminal application of 918 or 1125  $\mu\text{g}$   $^{14}\text{C}$ -monensin/day, radioactivity in milk amounted to 43-48  $\mu\text{g}/\text{kg}$ , but parent monensin represented only approximately 2% of this total label. These values may, however, exceed the established MRL value for milk of 2  $\mu\text{g}/\text{kg}$  monensin A (EMEA, 2007).

### 5.2.2. Other non-target animals

Kinetic data for other non-target animal species under consideration are not available.

### 5.3. Common drug-drug interactions

A common characteristic of ionophoric polyethers is their pharmacokinetic interaction with the veterinary drug tiamulin, which may lead to severe clinical symptoms in farm animals such as turkeys and chicken (Ratz *et al.*, 1997). The mechanisms of such interactions have been investigated in rats (Szucs *et al.*, 2004) and pigs (Witkamp *et al.*, 1994). Tiamulin has a dual effect on cytochromes P450 activity via the induction and/or the direct inhibition of CYP3A enzymes, which are involved in monensin O-demethylation. The inhibitory properties of tiamulin slow down the metabolic inactivation of monensin, resulting in a toxic syndrome

of apparent monensin overdosing (Szucs *et al.*, 2004). Substrate competition between monensin and chloramphenicol, erythromycin, oleandomycin and furazolidone, all being substrates for CYP3A enzymes, have been described as well, influencing the rate of excretion of either drug, but not resulting in clinical signs of toxicity (EFSA, 2004).

## **6. Risk characterization**

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

The available data from clinical cases of intoxications, tolerance studies and toxicological investigations in domestic animals others than the target animal species, indicate significant species differences in the susceptibility to monensin. In horses, which are generally known to be susceptible to ionophoric compounds, fatal intoxications were observed when they were fed monensin at a level of 121 mg/kg feed. Severe intoxications were also observed in sheep and goats at levels in feed that were lower than the maximum authorised level in complete feeds for chickens and turkeys (40-50 and 55 mg/kg respectively). Ducks also showed signs of toxicity at concentrations close to this level (160 mg/kg feed). In contrast, pigs showed a higher tolerance to the concentrations of monensin that are authorised for feeds intended for the target species.

In conclusion, accidental ingestion of feed intended for turkeys or chickens containing monensin at the maximum authorised level of 120 and 125 mg/kg feed, respectively, presents a health risk for several non-target animal species.

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

The highest concentration of monensin sodium that has been found in feed materials intended for non-target animals was 2.46 mg/kg feed. The levels of monensin are therefore expected to be below the level of 12.5 mg/kg feed that would result from a 10% cross-contamination of the maximum authorised level for chickens for fattening. At 12.5 mg/kg feed an average feed intake of 50 g/kg b.w. per day, applicable to most monogastric animal species, would lead to a daily intake of 0.63 mg monensin/kg b.w., which is above the overall NOAEL of 0.3 mg/kg b.w. (based on oral toxicity in dogs and rabbits). The same calculation for feed cross-contaminated at a level of 5% cross-contamination of the maximum authorised level for chickens for fattening would give a daily intake of 0.31 mg/kg b.w. which approaches the NOAEL. Therefore, the Panel concluded that adverse health effects in non-target animals may occur if cross-contamination of feed exceeds a level of 5% of the maximum authorised level for chickens for fattening.

### **6.3. Residues of monensin in foods derived from non-target animal species**

Animal studies suggested that monensin is rapidly absorbed, metabolised and excreted.

Residues of monensin equivalents (total radioactive residues) were measured in cattle for fattening that had been given feed containing 33 mg/kg of <sup>14</sup>C-labelled monensin sodium (see section 5.2.1). By linear extrapolation, a concentration of 25, 63 and 130 µg monensin equivalents/kg liver would be anticipated if the cattle had been given feed cross-contaminated at a level of 2, 5 and 10% (2.5, 6.25 and 12.5 mg/kg) of the maximum authorised level for chickens for fattening, respectively.

Data on monensin residues in animal-derived food products are available from the monitoring system according to Directive EC 96/23, from extensive surveillance studies conducted in the UK over several years and from market studies as described in chapter 3.2. Residues have been found in liver and eggs with a maximum concentration of 56 µg/kg in sheep liver and a maximum of 10 µg/kg found in eggs.

Parent monensin has not been reported from the surveillance in other animal tissues or milk.

### **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 values for daily human food consumption relevant for calculation of human exposure to monensin from cross-contaminated feed are 100 g of liver and 100 g of eggs<sup>21</sup>.

Data for residues of monensin from the 11-year UK survey (UK-VMD, 1995-2005) showed that the highest residue levels were found in a sheep liver sample (56 µg/kg). A person eating 100 g of this liver would be exposed to 5.6 µg of monensin (0.093 µg/kg b.w. for a 60 kg person), which is approximately 3% of the ADI of 3 µg/kg b.w. The contribution from the highest concentration found in eggs (10 µg/kg) would be 1 µg monensin (0.017 µg/kg b.w. for a 60 kg person), which is 0.6% of the ADI. These results indicate that the actual residue levels will almost always be considerably lower than the theoretical estimates based on kinetic data.

At the predicted concentration of monensin equivalents in cattle liver tissue resulting from 10% cross-contamination (130 µg monensin equivalents/kg liver), such consumption would lead to exposure to 13 µg monensin equivalents per person per day (corresponding to 0.22 µg/kg b.w. per day for a 60 kg adult). This consumption represents 7.3% of the ADI.

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<sup>21</sup> Values for daily human food consumption, as defined in Directive No (EC) 2001/79 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

On the basis of experimental data on chickens, the FEEDAP panel calculated the total consumer intake from target animal tissues at zero-withdrawal time to be 287 µg/person (EFSA, 2006c). Assuming the kinetics and tissue distribution is the same for laying hens compared to chickens, and assuming a cross contamination of 2, 5 and 10 % of the maximum authorised level of monensin (corresponding to 2.5, 6.3 and 12.5 mg/kg feed) it is estimated by linear extrapolation that consumers would be exposed to monensin at levels of approximately 6, 14 and 29 µg/person (0.1, 0.2 and 0.5 µg/kg b.w.) corresponding to 3, 8 and 16% of the ADI from edible tissues derived from laying hens. Therefore, it is concluded that there is minimal consumer risk from eating muscle, liver, kidney or skin/fat from laying hens that have been fed cross-contaminated feed containing up to 10% cross-contamination of monensin sodium. In addition the likelihood of exposure from tissues of laying hens is low.

The above estimates are well below the ADI of 3 µg/kg b.w as derived by the FEEDAP Panel. In addition, it was recognised that consumer exposure to residues of monensin resulting from cross-contamination of feed is likely to be infrequent.

Therefore, the CONTAM Panel concludes that there is negligible risk to consumers' health from the ingestion of monensin residues in animals exposed to feed cross-contaminated feed up to a level of 10% of the maximum authorised level for chickens for fattening.

## CONCLUSIONS

- Monensin exerts signs of toxicity typical of ionophoric compounds in various non-target animal species. Comparison of the available toxicological data suggests that horses are particularly sensitive, and dogs, small ruminants and ducks are very sensitive species, whereas pigs are less susceptible to monensin. Intoxications in these animal species, particularly in young animals, can be fatal, and may occur in sensitive animal species at feed concentrations in the range of the maximum level authorised for use in chickens and turkeys.
- Adverse health effects in sensitive non-target animal species may occur as a result of exposure to feed batches cross-contaminated at a level exceeding 5% of the maximum authorised monensin concentration. At this contamination level the potential exposure of 0.31 mg/kg b.w. per day is approaching the overall NOAEL of 0.3 mg/kg b.w. per day derived from toxicological studies in dogs and rabbits.
- The available kinetic data show that monensin residues primarily occur in the liver, but are also occasionally found in eggs at low levels.
- Calculations based on the experimental kinetic data indicate that the maximum level of human exposure from monensin residues (total radioactive residues) would be 0.22 and 0.28 µg/kg b.w. per day from cattle and chicken liver, respectively, if feed is cross-contaminated at a level of 10% of the maximum authorised level for chickens for fattening. The available surveys conducted by Member States indicate that the actual levels of monensin found in liver are lower.
- Human exposure estimated to result from consumption of food products from non-target animal species exposed to cross-contaminated diet is well below the ADI of 3 µg/kg b.w.. Therefore, the Panel concludes that there is negligible risk to consumers' health from the ingestion of monensin residues in products from animals exposed to feed cross-contaminated up to a hypothetical level of 10% of the maximum level authorised for chickens for fattening.

## RECOMMENDATIONS

- Sensitive analytical methods that have become available for the detection of monensin in animal products should be validated also for feed concentrations below the maximum authorised level, to assess their applicability in the control of cross-contamination of feed batches during the production process.
- Analysis of milk samples for residues of monensin should be encouraged as no data are available at present.

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Eli Lilly & Co. Ltd, 2006. Safety of monensin in non-target species. Confidential report.

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## **Occurrence data**

Belgium. AFSCA, The Food Agency.

Czech Republic. Central Institute for Supervising and Testing in Agriculture.

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

European Commission, DG SANCO.