

**Cross-contamination of non-target feedingstuffs by maduramicin 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-2005-220F**

**Adopted on 26 November 2007**

**PANEL MEMBERS**

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**SUMMARY**

Maduramicin ammonium is a polyether carboxylic ionophore agent that is authorised according to Commission Regulation No (EC) 1464/2004 as a coccidiostat for use in chickens for fattening and turkeys (until a maximum age of 16 weeks) with a maximum content of the active substance in feed of 5 mg/kg and a withdrawal period of five 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 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 potential residues in foods derived from these non-target animal species have been evaluated.

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<sup>1</sup>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 maduramicin authorised for use as a feed additive, *The EFSA Journal* (2008) 594, 1-30

Species differences in the susceptibility to maduramicin and the small margin of safety between the maximum authorised level and the minimum toxic dose in non-target animal species have been shown. Signs of intoxication have been reported in rabbits, cattle and sheep at the maximum authorised level in chickens for fattening and turkeys (5 mg/kg). The toxic syndromes include cardiomyopathy associated with congestive heart failure and mild to severe skeletal muscle lesions, consistent with the mode of action of polyether ionophores. In turkeys, depression of body weight gain was seen above 10 mg/kg feed and lethal effects were observed in pigs at 37.5 mg/kg feed. The Panel on Contaminants in the Food Chain (CONTAM Panel) concluded that accidental ingestion of the maximum authorised level of maduramicin in feed for chickens for fattening and turkeys may present a health risk for several non-target animal species.

Cross-contamination of feed at a level of 10% (0.5 mg/kg feed) of the maximum authorised maduramicin concentration, would result in an intake for non-target animal species of up to 0.025 mg/kg b.w. per day of maduramicin. This level is below the overall no observed effect level (NOEL) of 0.16 mg/kg b.w. as identified by the Scientific Committee on Animal Nutrition (SCAN) from a two year oral (feeding) study in rats, and below the lowest observed adverse effect level (LOAEL) of 0.075 mg/kg b.w. per day of the most sensitive species, the rabbit. The CONTAM Panel concluded that adverse health effects are unlikely to occur in non-target animals as a result of cross-contamination of feed at a level up to 10% of the maximum authorised level of maduramicin in feed for target animals.

No kinetic or occurrence data were available to estimate the amount of maduramicin residues in eggs, milk or in meat and offal from non-target animal species. Hence, consumer exposure was estimated using kinetic data at zero withdrawal time from chickens for fattening that were given the maximum authorised level of 5 mg maduramicin/kg feed. These data were extrapolated to a concentration of 0.5 mg/kg feed to correspond to feed cross-contaminated with maduramicin at a level of 10% of the maximum authorised level. Consumption of such poultry products (100 g of liver, 300 g muscle, 90g skin/fat and 10g of kidney) could give an intake of 2.2 µg maduramicin equivalents (total radioactive residues) per person corresponding to 0.037 µg/kg b.w. for a 60 kg consumer, which represents 3.7% of the ADI of 1 µg/kg b.w. per day as established by the SCAN. Even though kinetic and tissue deposition can differ between chickens for fattening and non-target animal species, an exceedance of the ADI is unlikely to occur. Therefore, the CONTAM Panel concluded that the very limited data provided no indication of an appreciable risk to consumers' health from the ingestion of maduramicin residues in products from animals exposed to feed cross-contaminated up to a hypothetical level of 10% of the maximum authorised level.

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

**TABLE OF CONTENTS**

BACKGROUND AS PROVIDED BY THE REQUESTOR.....	4
1. Cross-contamination .....	4
2. Legal provisions as regards minimisation of cross-contamination.....	4
3. Legal provisions as regards the authorisation of coccidiostats (and histomonostats) for use as feed additive.....	5
4. Unavoidable cross-contamination (under practical conditions) .....	6
5. Tolerances .....	6
TERMS OF REFERENCE AS PROVIDED BY THE REQUESTOR.....	7
ACKNOWLEDGEMENTS .....	7
GLOSSARY OF TERMS USED BY THE PANEL IN ITS OPINIONS ON COCCIDIOSTATS .....	8
ASSESSMENT .....	10
1. Introduction.....	10
1.1. Biological activities of maduramicin.....	11
1.2. Previous evaluations of the toxicological properties and the safety of maduramicin .....	12
1.3. Cross-contamination of feed batches.....	13
1.4. Specific data for maduramicin ammonium-based feed additive products .....	15
2. Methods of analysis for maduramicin.....	15
2.1. Analysis of maduramicin in premixes and animal feeds.....	15
2.2. Analysis of maduramicin residues in animal products.....	15
3. Occurrence of maduramicin.....	17
3.1. Occurrence of maduramicin residues in feed materials for non-target animal species .....	17
3.2. Occurrence of maduramicin residues in animal products derived from non-target animal species .....	17
4. Toxicity of maduramicin.....	18
4.1. Mechanisms of toxicity .....	18
4.2. Toxicity of maduramicin in target animal species .....	18
4.3. Toxicity of maduramicin in non-target animal species .....	19
5. Kinetics and tissue distribution.....	23
5.1. Kinetics of maduramicin in the target animal species .....	23
5.2. Kinetics of maduramicin in non-target animal species .....	25
6. Risk characterization.....	25
6.1. Animal health risks in non-target animal species associated with the accidental consumption of feed materials designated for target animal species .....	25
6.2. Adverse health effect in non-target animal species as a consequence of cross- contamination of feed batches.....	25
6.3. Residues of maduramicin in foods derived from non-target animal species.....	26
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.....	26
CONCLUSIONS .....	27
RECOMMENDATIONS .....	28
REFERENCES .....	28
DOCUMENTATION PROVIDED TO EFSA .....	30

## **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 following 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 (EC) No 95/69**

Directive (EC) No 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,<sup>2</sup> provides in Article 2 and 3, that establishments manufacturing coccidiostats, manufacturing premixtures prepared from coccidiostats, manufacturing compound feedingstuffs containing

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<sup>2</sup> OJ L 332, 30.12.1995, p. 15. As last amended by Council Regulation EC° 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 (EC) No 183/2005**

Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene<sup>3</sup> was applicable from 1 January 2006 onwards and has replaced Council Directive (EC) No 95/69.

Article 10 of Regulation (EC) No 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 (EC) No 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 (EC) No 70/524 concerning additives in feedingstuffs<sup>4</sup> 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

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

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

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.

Maduramicin ammonium has latest been assessed by the Scientific Committee for Animal Nutrition (SCAN) (EC, 1988,1997) and has been authorised for use as feed additive in accordance with the provisions of Council Directive 70/524/EEC (see Table 1).

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 (EC) No 70/524 with effect from that date.

Table 1. Species or category of animals for which the use of maduramicin ammonium 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)</b>	<b>Authorised maximum content of active substance in complete feed</b>
Maduramicin ammonium	Chickens for fattening Turkeys (max. 16 weeks)	5 mg/kg (Cygro) 5 mg/kg (Cygro)

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

Maduramicin ammonium is authorised for use as a feed additive for the production of feedingstuffs for target species according to the conditions of authorisation. However the production of feed containing maduramicin ammonium can result in cross-contamination to feedingstuffs for non-target 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 batches, the cross-contamination of residues is unavoidable under practical conditions.

#### **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>.

<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)

Such tolerances in feedingstuffs for non-target 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 of 5% and below can be achieved after implementing severe actions to reduce cross-contamination.

### **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 maduramicin ammonium 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 maduramicin ammonium 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 maduramicin ammonium into feed from 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.
- 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**

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 (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 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. However, none of the compounds under consideration are licensed at present as veterinary medicinal product. The FEEDAP has also recommended MRLs for some coccidiostats, and the CONTAM Panel considered these recommendations in the evaluation process.

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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).

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

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

**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 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

Maduramicin ammonium is a salt of a polyether carboxylic acid ionophore that was originally isolated from a culture of *Actinomadura yumaensis*. It exerts both anticoccidial and antibacterial effects. The maduramicin ammonium that is used as a feed additive (Cygro) consists of 90% of the ammonium salt of a polyether monocarboxylic acid with an -OCH<sub>3</sub> group at the C5 position of the A ring (alpha-maduramicin) and 10% of the structurally similar compound with an -OH group instead of -OCH<sub>3</sub> at the C5 position of the A ring (beta-maduramicin). Alpha-maduramicin has a preferential affinity for monovalent cations as was shown in ion displacement studies (EC, 1988). The chemical structure of alpha-maduramicin ammonium is presented in Figure 1.

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

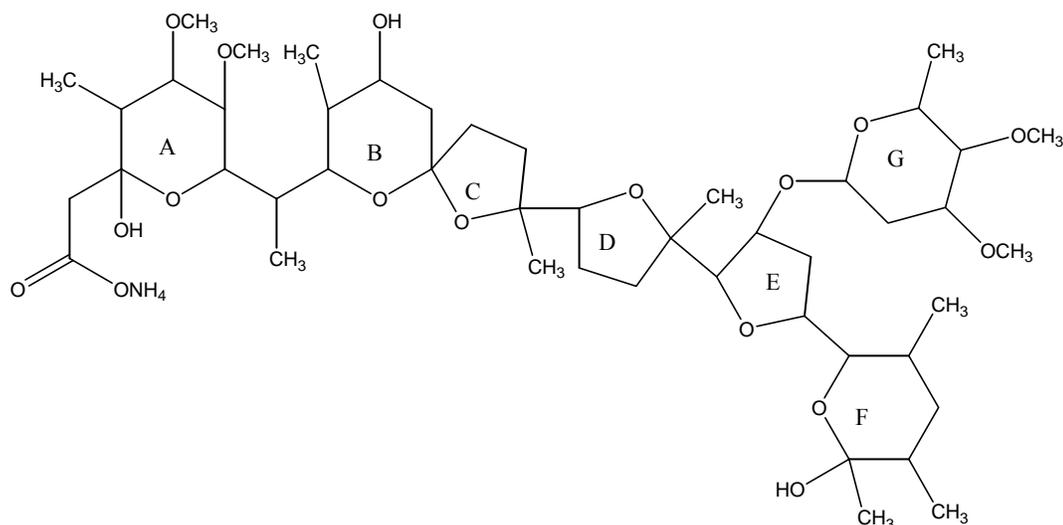


Figure 1. Chemical structure of alpha-maduramicin ammonium

The IUPAC name of alpha-maduramicin ammonium is 2-[(2R,3S,4S,5R,6S)-6-[(1R)-1-[(2S,5R,7S,8R,9S)-2-[(2R,5S)-5-[(2R,3S,5R)-3-[(2R,4S,5S,6S)-4,5-dimethoxy-6-methyl-oxan-2-yl]oxy-5-[(2S,3S,5R,6S)-6-hydroxy-3,5,6-trimethyl-oxan-2-yl]oxolan-2-yl]-5-methyl-oxolan-2-yl]-9-hydroxy-2,8-dimethyl-1,6-dioxaspiro[4.5]dec-7-yl]ethyl]-2-hydroxy-4,5-dimethoxy-3-methyl-oxan-2-yl]acetic acid and the CAS number is 84878-61-5. The molecular weight is 934 and the molecular formula is  $C_{47}H_{83}NO_{17}$ .

Maduramicin ammonium is a white or almost white crystalline powder with a characteristic odour. It is freely soluble in trichloro-methane, methanol, ethanol and acetone but insoluble in water.

As summarised in the background chapter, maduramicin ammonium is authorised as a coccidiostat for chickens for fattening (Regulation (EC) No 2430/1999<sup>11</sup>) and turkeys (max. 16 weeks) (Regulation (EC) No 2380/2001<sup>12</sup>) at a minimum/maximum content of active substance in complete feed of 5 mg/kg. The withdrawal period is 5 days.

## 1.1. Biological activities of maduramicin

### Effect on coccidia

Maduramicin is considerably more potent as a coccidiostat than the other polyether ionophores that are used as coccidiostatic feed additives. These compounds are branch-chained, polyoxygenated carboxylic acids that act as mobile carriers of cations by rendering

<sup>11</sup> OJ L 296, 17.11.1999, p.3-11.

<sup>12</sup> OJ L 321, 6.12.2001, p. 18-19.

cations lipid-soluble, thereby enabling them to pass across membranes. This process disrupts cationic cross-membrane gradients and is responsible for their anticoccidial activity.

### **Antibacterial activity**

As other polyether ionophores, maduramicin ammonium possesses moderate activity against many gram-positive bacteria, but with no activity against gram-negative organisms. Although no *in vitro* studies on the development of resistance and cross resistance were carried out, *in vivo* studies showed that stable resistance caused by chromosomal mutation in sensitive gram-positive organisms did not develop, even though a transient loss in sensitivity was observed. Maduramicin did not produce the selection of transmissible resistance factors in indigenous faecal coliforms nor in experimentally introduced *Salmonella*. No effect was observed on colonisation or shedding of *Salmonella* in chickens (EC, 1988).

The addition of maduramicin ammonium at the dose of 5 mg/kg to complete feedingstuff for chickens for fattening does not lead to the development of bacterial resistance to prophylactic or therapeutic preparations nor does it cause recurrent infections of gram-negative bacteria in the gut of chickens for fattening (EC, 1988).

The effect of maduramicin on the bacterial populations that make up the human gut flora has not been evaluated and a microbial ADI has not been established.

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

A toxicological assessment of maduramicin has been performed by the Scientific Committee for Animal Nutrition (SCAN) in 1988 and 1997 (EC, 1988, 1997). However, the data submitted to the SCAN by the applicant were not available to the CONTAM panel. SCAN evaluated toxicological studies in mice, rats, rabbits and dogs. The toxicological studies investigated irritancy, acute toxicity; repeat dose toxicity over 28-days, 90-days and 12-months; chronic toxicity; mutagenicity, carcinogenicity; and reproductive toxicity, including multigeneration and developmental toxicity studies. Maduramicin was reported to be irritating to the skin and corrosive to the eye. No information was available on its allergenic potential. The alpha and beta forms of maduramicin had high acute oral toxicity in the mouse and rat and a high dermal toxicity in rabbit but no doses were reported in the SCAN opinion. Subchronic studies in rats showed adverse effects on growth, the heart being the main target organ (LOEL 0.35 mg/kg b.w.), The heart, skeletal muscle and eye were reported being as target organs in dogs (LOEL = 0.35 mg/kg b.w.), but details of the effects were not reported by SCAN. Pharmacological studies in rats and dogs showed “no evidence of myocardial damage”, but ECG changes were seen in dogs at 1 mg/kg b.w. (the route of administration was not reported by SCAN). Maduramicin was not shown to be teratogenic or fetotoxic in

developmental toxicity studies, although a dose of 3 mg/kg b.w. per day caused 100% maternal mortality (species not specified). Marginal effects on pup weight offspring size and pup survival at 0.15 mg/kg b.w. per day were seen in the F<sub>2</sub>b-generation of a rat multigeneration reproduction study. Maduramicin was reported as giving an equivocal result in an *in vitro* test for chromosomal aberrations in mammalian cells, but it was said to be negative in other (unspecified) mutagenicity tests and was not shown to be carcinogenic in rats (EC, 1988). The SCAN derived an ADI of 1 µg/kg b.w. for maduramicin based at the no-effect level (NOEL) of 0.16 mg/kg b.w. per day from a two-year rat oral toxicity study, applying a uncertainty factor of 100 and rounding down to one-significant figure. The SCAN did not report what toxicological effects had been seen at doses greater than the NOEL in the two-year rat study.

In contrast to some other ionophoric polyethers, regulatory authorities have not established a microbiological ADI value for maduramicin.

### 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 (see also the background chapter) and may contaminate the 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; 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 premixes.

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 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 (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).

The 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 for lasalocid, narasin, nicarbazine and monensin (Kennedy *et al.*, 1996, 1998; Mc Evoy *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.

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

In order to determine the stability of maduramicin in complete feed for chickens for fattening, three standard diets containing nicarbazine and maduramicin were prepared. The feeds were assayed before and after storage for two months at 25°C. Maduramicin recovery was on average 89.4% (Alpharma, Gromax Technical Manual).

No additional information has been identified for maduramicin ammonium concerning cross-contamination and physical parameters.

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

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

No methods were identified which would meet the sensitivity requirements (LOQ 50 µg/kg) to be applicable to quantify maduramicin in cross-contaminated feeds for non-target animal species.

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

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),

maduramicin residues methods are reported for muscle or liver tissues by 3 and for eggs by 4 out of the 20 NRLs within the EU. The Member States used different methods such as liquid chromatography–mass spectrometry (LC-MS) or liquid chromatography – tandem mass spectrometry (LC-MS/MS) for screening and confirmatory purposes. The decision limits ranged between 0.3 and 100 µg maduramicin/kg tissue.

No maximum residue limit (MRL) or minimum required performance level (MRPL) has been established for maduramicin in eggs or animal tissues.

### **2.2.1. Screening methods**

Enzyme-linked immunosorbent assay (ELISA) has been used as screening technique for coccidiostats. Kennedy *et al.*, (1997) developed an ELISA method for screening coccidiostats in poultry meat. They reported that the antibodies used exhibited slight cross-reactivity with structurally similar ionophoric coccidiostats. For maduramicin the limit of detection (LOD) was estimated to be 0.02 µg/kg in tissue and the limit of quantification (LOQ) 1.0 µg/kg in tissue (Kennedy *et al.*, 1997). An ELISA method with immunoaffinity clean-up for the analysis of maduramicin in chicken tissues was developed by Shen *et al.* (2001). LODs for maduramicin were reported to be 1 µg/kg, 2.8 µg/kg and 1.5 µg/kg in muscle, liver and fat, respectively (Shen *et al.*, 2001).

### **2.2.2. Quantitative and confirmatory methods**

Dubois and colleagues (2004) described a qualitative multi-residue LC-MS/MS method to determine nine coccidiostats in muscle and chicken eggs. Maduramicin was extracted into acetonitrile followed by a clean-up with solid phase extraction. Two mass transitions ( $m/z$  939.8 → 877.5 and  $m/z$  939.8 → 719.5) were monitored. For maduramicin residue in muscle, extraction recovery was 52% and  $CC\alpha^{13}$  was 0.4 µg/kg.

Jestoi and colleagues (2007) described a quantitative multiresidue LC-MS/MS method to determine ionophoric coccidiostats including maduramicin in poultry tissues (liver and

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<sup>13</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).

muscle). Two mass transitions ( $m/z$  939  $\rightarrow$  877 and  $m/z$  939  $\rightarrow$  895) were monitored. For maduramicin residue in muscle and liver, CC $\alpha$  were 2 and 2.1  $\mu\text{g}/\text{kg}$ , respectively.

### **3. Occurrence of maduramicin**

#### **3.1. Occurrence of maduramicin 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. None of the samples contained maduramicin above the LOD of 0.5 mg/kg (data provided to EFSA).

Information from the Rapid Alert System for Food and Feed (RASFF)<sup>14</sup> that was collected between April 2002 and April 2006 showed no incidents of maduramicin in feed for non-target animal species (data provided by the European Commission).

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

Residues of maduramicin in non-target 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 animal species are analysed for residues of coccidiostats by the Member States according to requirements in Directive (EC) No 96/23<sup>15</sup>. However, the results from the 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  $\mu\text{g}$  maduramicin/kg tissue as their non-compliant limit.

For maduramicin, combined results of 2004 and 2005 show that five non-compliant samples were found amongst 4424 samples of different animal tissues. Three non-compliant samples were found in poultry (2 out of 971 in muscle and 1 out of 314 in liver) analyses, one non-compliant sample was found in eggs out of 1319 samples analysed and one non-compliant sample was found in bovine liver out of 48 samples analysed. The LOD ranged from 0.35 to 100  $\mu\text{g}/\text{kg}$  (data provided by the European Commission).

Belgium has provided individual data for 1600 samples of foods that were analysed for maduramicin between 2004 and 2006. Two samples, one egg and one poultry muscle,

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<sup>14</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>15</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.

contained residues of maduramicin that were greater than the Belgian defined non-compliant limit of 10 µg/kg. One sample of poultry muscle contained a concentration of maduramicin that was greater than the LOD of 2 µg/kg but less than and the Belgian non-compliant limit of 10 µg/kg (data provided to EFSA).

There were no analyses for maduramicin in the UK surveys for residues in foods over the period 1995-2005.

#### **4. Toxicity of maduramicin**

##### **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. Ionophores also differ in molecular polarity, which affects their differential distribution in biological membranes.

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

###### **4.2.1 Chickens for fattening**

Administration of maduramicin to chickens for fattening at a level of 15 mg/kg feedingstuff resulted in significant depression of body weight gain. Feed efficiency was reduced at doses from 8 - 15 mg/kg feedingstuff. No other treatment related effects were noted except a slight reduction in lymphoid tissue of the bursa and thymus at 15 mg/kg (EC, 1988). No NOAEL was identified.

More recently, experimental chickens were given maduramicin in medicated feed at 5 and 10 mg/kg for 21 days and such treatment caused growth retardation from 14 days onwards at both doses with clinical signs such as watery diarrhoea, depression, dullness and ruffled feathers from the second week in birds given 10 mg/kg and from the third week in the birds given 5 mg/kg. No toxic effects were seen after seven days of treatment at both doses. There was a significant reduction in haemoglobin in both medicated groups from day 14 and total erythrocyte count and packed cell volume in the 10 mg/kg group on day 21. An increase in mean corpuscular volume (MCV) in 10 mg/kg group on day 21 indicated macrocytic anaemia and leucopenia due to lymphopenia was also observed. A decrease in mean corpuscular haemoglobin concentration in both the medicated groups indicated hypochromic anaemia. Overall, the authors concluded that maduramicin is safe to use for 7 days at both 5 and 10 mg/kg feed levels but that toxic effects will occur at both levels from day 14 (Singh and Gupta, 2003).

#### **4.2.2. Turkeys**

An experiment was conducted to determine the clinical safety of 5 mg maduramicin/kg of complete feed in turkeys of different ages. Turkeys were assigned to group in pens of 15 males or 17 females. At days 1, 5, 29, 43, and 57, four pens of males (60) and females (68) were transferred from the control diet to one containing maduramicin for 2 days. Throughout the study there was no difference in performance parameters between the turkeys recently introduced to maduramicin and the control group.

Another study carried out involved 24 male and 24 female turkeys (20 weeks old). They were fed diets containing maduramicin at 5, 10 or 15 mg/kg of complete feed for 2 weeks. There were no apparent adverse effects with maduramicin at 5 mg/kg of complete feed. The maduramicin at 10 mg/kg of complete feed had pronounced effects on the daily weight gain of the male turkeys, but females were unaffected. There was no mortality or signs of clinical adverse effects. At 15 mg/kg, 5 turkeys died after an average of 8 days but the daily weight gain of the survivors was unaffected. It was concluded that maduramicin at 5 mg/kg of complete feed is safe when introduced to turkeys at any time during the growing period (EC, 1997).

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

#### **4.3.1. Laying poultry**

Groups of 8 White Leghorn hens (58 weeks old) received maduramicin ammonium at doses of 0, 2.5 and 5 mg/kg of feed. There were no statistically significant differences between controls and the two medicated groups for feed consumption, egg production or egg shell thickness. Maduramicin at 5 mg/kg significantly increased Haugh units (viscosity index) and decreased yolk colour during the medication period when compared to the control and the group receiving 2.5 mg/kg. No mortality or clinical changes were recorded during the study (data provided by industry).

Another study in leghorn breeders compared egg production, feed consumption, egg weights, fertility, shell thickness, yolk colour, hatching rate, dead embryos and pips in a control group of a group receiving maduramicin ammonium at doses of 5 mg/kg of feed. The only effect observed was a statistically significantly higher number of pips at hatching and there were no effects on egg production (data provided by industry).

For laying turkeys, the SCAN report concluded that maduramicin at 5 mg/kg feed did not have any adverse effects, no details are given (EC, 1988).

#### **4.3.2. Guinea fowl**

Maduramicin at 5 mg/kg feed was reported to have no adverse effects on guinea fowl, no details were given (EC, 1988).

#### **4.3.3. Rabbits**

Six groups of 32-day old rabbits received maduramicin at doses of up to 5.0 mg/kg of feed (equivalent to 0.15 mg/kg b.w. per day) for 40 days (data provided by industry). The endpoints measured included growth rate, feed intake, mortality and general clinical observations. Weight gain and feed intake were measured at 20 and 40 days of the study. Maduramicin ammonium did not significantly affect the food intake, weight gain or feed conversion ratio. There was no mortality, clinical signs or abnormal behaviour during the study. The authors concluded that maduramicin ammonium is safe to rabbits at levels up to 5 mg/kg in the feed.

The SCAN report concluded that maduramicin at 5 mg/kg feed did not have any adverse effects on fattening rabbits, this conclusion is based on a dossier provided by the applicant and no details are given in the report (EC, 1988).

A group of 30 rabbits for fattening (32-day old) received maduramicin at doses of 0, 1, 2, 3, 4, 5 or 6 mg/kg of feed for 40 days (equivalent to 0, 0.03, 0.06, 0.09, 0.12, 0.15 or 0.18 mg/kg b.w. per day) (data provided by industry). Lower weight gain, lower feed intake, and higher mortality of animals fed with maduramicin at doses of 3 mg/kg and higher were reported. At the dose levels of 1 and 2 mg/kg feed conversion rate was more efficient than in controls, with similar final body weight and mortality. All exposure groups consumed less feed than non medicated animals. It was concluded by the authors that more than 2 mg maduramicin/kg feed (0.06 mg/kg b.w. per day) would adversely affect the productivity or the health of the animals.

Groups of 5 and 10 rabbits (6 to 7 weeks old) were fed with maduramicin at doses of 0, 2.5, 5, 7.5 or 10 mg/kg of feed for two weeks (equivalent to 0, 0.075, 0.15, 0.225 or 0.3 mg/kg b.w.) (data provided by industry). At the lowest dose, mortality was not observed but a slightly increased rate of growth was observed. At 5 mg/kg in the diet, 4 out of 10 rabbits died after two weeks of exposure. At 7.5 mg/kg, 4 out of 5 rabbits died after eight days and in the 10 mg/kg group, all rabbits died during the first week. A LOAEL of 0.075 mg/kg b.w. per day could be derived from this study.

Overall, these studies in rabbits show that maduramicin is toxic at levels corresponding to the maximum authorised level for target animals in feed of 5 mg/kg (0.15 mg/kg b.w per day), no toxicity was observed at 2.5 mg/kg (0.075 mg/kg b.w per day).

#### **4.3.4. Pigs**

Pigs that consumed feed which contained maduramicin ammonium at the dose levels of 2.5 or 5 mg/kg of feed (corresponding to 0.075 or 0.15 mg/kg b.w.) gained more weight, consumed more feed and had a better feed conversion rate than the control animals (data provided by industry). There were no mortality or toxic signs in the exposed animals.

Maduramicin at 5 mg/kg feed was reported to have no adverse effects on grower or finishing pigs (EC, 1988).

The death of 550 weaned, 6 - 12-week-old pigs was reported from an Ontario farm and shown to be due to the accidental feed of maduramicin to the pigs at a high dose (37.5 mg/kg) mislabelled as oxytetracycline. Clinical signs were anorexia, respiratory distress, lethargy, ataxia, recumbency, unwillingness to stand even when assisted, and death. Degenerative changes included swelling, eosinophilia and loss of cross-striations of myofibres, and coagulation necrosis, fragmentation and formation of retraction caps in more severely damaged areas of the muscles. Values for aspartate aminotransferase and creatine kinase were also greatly elevated in sera from five pigs tested (Sanford and McNaughton, 1991).

#### **4.3.5. Horses**

Three groups of four horses (geldings and mares), received maduramicin at a dose level of 0, 5, or 10 mg/kg in the diet (equivalent to 0, 0.01 or 0.2 mg/kg b.w.) for 14 days (data provided by industry). Body weight, daily feed consumption and blood chemistry were measured. No symptoms or decrease in feed consumptions were observed during the 14 days of treatment which indicated that 0.01 or 0.2 mg/kg b.w. can be considered safe for horses.

Maduramicin at 5 mg/kg feed was reported to have no adverse effects on horses (EC, 1988).

#### **4.3.6. Ruminants**

Six Holstein cows in mid-lactation received maduramicin at dose levels of 0 or 4 mg/kg of diet (equivalent to 0 or 0.06 mg/kg b.w. per day) for 14 days (data provided by industry). The end-points measured included daily milk production and feed consumption recorded during a pre-treatment week, during two weeks of treatment and a post treatment week. There were no symptoms of toxicity during the treatment and post treatment periods. There were no significant differences in the milk production and the feed consumption.

A group of steers received maduramicin for 28 days at 5 mg/kg of feed (equivalent to 0.15 mg/kg b.w. per day). The feed was prepared, by mixing two parts of non-medicated steer concentrate with one part of medicated poultry diet (data provided by industry). All steers

received the same amounts of non-medicated feed for 28 days. The average daily body weight gain and feed conversion rates were the same in exposed and control groups after 28 days. Maduramicin at a dose of 0.15 mg/kg b.w. per day was considered safe for use in steers.

An acute toxicosis has been described (Shlosberg *et al.*, 1997) due to accidental contamination of feed with maduramicin and monensin in calves, in which sudden death was in most cases the sole clinical finding. The mortality of the herd of 277 male beef calves was 51% mortality during 40 days in the group aged 5 - 8 month (212 calves) and, 11% mortality was reported in the other two groups aged 9 – 11 months (41 calves) and 15-16 months (24 calves) over the 40-day period. At the time of the toxicosis, all the groups were fed the same diet, a nutritionally balanced total mixed ration consisting mainly of 48% ground barley and 24% ground corn. Analysis revealed the exposure of the calves to monensin and maduramicin in the total mixed ration at 12 mg/kg and up to 6 mg/kg, respectively. No evidence could be found of whether the resultant toxicity might have been additive or synergistic (Shlosberg *et al.*, 1997).

Reports are available from outbreaks of poultry litter<sup>16</sup> toxicity in South Africa (20 cattle and 4 sheep from 15 outbreaks) resulting from batteries where maduramicin that was incorporated into rations of broilers. The poultry litter was fed *ad libitum* to the affected stock as 30 – 80% by volume of their rations and the main clinical sign manifested was sudden mortality of up to 70% of the herd, usually within 20 - 40 days of exposure to the poultry litter. A number of cattle developed signs of congestive heart failure, and stiffness, tachycardia and/or cardiac arrhythmia, together with the elevated activity of aspartate transaminase (AST) and/or lactate dehydrogenase (LDH) in the sera. Analysis by HPLC of poultry litter implicated in toxicity in cattle in 2 of the outbreaks revealed that they contained 2.5 mg/kg and 6.1 mg/kg of maduramicin (Fourie *et al.*, 1991).

The same authors published another case report in which the gross and histopathological examinations of 20 cattle in field outbreaks of toxicity due to feeding with poultry litter and 1 steer fed *ad libitum* with poultry litter were carried out (Bastianello *et al.*, 1995). In most of the cattle that died in field outbreaks, the principal macroscopic lesions were indicative of congestive heart failure. Microscopically, the cardiac lesions comprised of various degrees of atrophy, hypertrophy, degeneration, necrosis of myocardial fibres and interstitial fibrosis. In the steer fed *ad libitum*, extensive hypertrophy and atrophy of myocardial fibres was seen.

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<sup>16</sup> Poultry litter consists of manure, feathers, spilled chicken feed, wood shavings, corn husks, straw, etc. that accumulate on the floors of the buildings in which broiler chickens are raised. It can contain disease-causing bacteria, antibiotics, heavy metals, restricted feed ingredients including meat and bone meal from dead cattle, and even foreign objects such as dead rodents, rocks, nails and glass. This material is collected, processed using techniques such as composting and deep stacking, and then added to cattle feed because of its high protein and mineral content (explanation taken from <http://www.fact.cc/humanecalfbkgr.htm>).

Maduramicin at 5 mg/kg feed was reported to have no adverse effects on lactating cows or finishing steers (EC, 1988).

A case report gave details of gross and histopathological lesions in four sheep in 15 field outbreaks due to consumption of poultry litter and 6 sheep dosed with poultry litter and 10 sheep fed experimental rations containing maduramicin at a dose of 2.5 or 5 mg/kg of feed (Bastianello *et al.*, 1995). The pathology in sheep was characterized by a cardiac dilatation, congestive heart failure with cardiomyopathy and severe hindquarters skeletal muscle lesions which appeared pale, oedematous and mottled.

#### **4.3.7. Human data**

A group of seven individuals in India had eaten a total of approximately 450 g of maduramicin (an average of about 65 g/person or approximately 1 g/kg b.w. per day corresponding to 1 million times the ADI) from a pudding mixed with vegetable oil. Within 4 hours all victims developed vomiting and weakness of all four limbs and truncal muscles. Two of the victims died within 2 days of rhabdomyolysis associated with hyperkalaemia, metabolic acidosis and hypocalcaemia, respiratory failure and polyneuropathy. In both cases, the victims had markedly elevated values for creatinine phosphokinase MM isoenzyme compared to surviving victims. At 8 days after exposure, the survivors were admitted to hospital. The clinical examination revealed toxic polyneuropathy with varying degrees of rhabdomyolysis. Electrocardiograph and echocardiograph results were normal and cardiomyopathy was not detected in any of the surviving patients. Nerve conduction studies showed polyradiculopathy. Pigment-induced acute renal failure developed at 8 - 10 days after exposure in 4 of the 5 survivors. Some patients needed respiration support for several days. Muscle pain subsided three weeks after exposure (Sharma *et al.*, 2005).

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

### **5.1. Kinetics of maduramicin in the target animal species**

The kinetics of maduramicin in chickens for fattening and turkeys has been evaluated by SCAN (EC, 1988, 1997). When compared to the chicken for fattening, the metabolic fate of maduramicin ammonium in the turkey appeared to be very similar in terms of biliary excretion, and the nature of the excreted metabolites and tissue residues. However, in the turkey, plasma and tissue residue levels were lower and the disappearance of the residues from the tissues, based on the same LOD of 25 µg/kg, was faster (3 days instead of 7 days).

### **5.1.1. Chickens for fattening**

Male chickens for fattening were given 5 mg unlabelled maduramicin/kg feed for 14 days. Serum, breast muscle and liver were collected 7 days after withdrawal. The half-life of maduramicin in serum was approximately 13 hours. In liver and muscle, the half-lives were 20 and 39 hours, respectively. Immediately after dosing (i.e. at zero withdrawal time), the highest mean maduramicin concentration was observed in liver (160 µg/kg) followed by serum (70 µg/L) and muscle (20 µg/kg). By linear extrapolation, a concentration of 3, 8 and 16 µg maduramicin/kg liver would be anticipated if the chickens had been given feed cross-contaminated at a level of 2, 5 and 10% (0.1, 0.3 and 0.5 mg/kg feed), respectively. The corresponding values for muscle are 0.4, 1 and 2 µg/kg. At the recommended withdrawal time of 5 days, the residual concentration in the liver, muscle and serum was 1.6, 1.5 µg/kg and 0.05 µg/L, respectively (Kennedy *et al.*, 1997).

### **5.1.2. Turkeys**

In turkeys (Stout *et al.*, 1991), chickens and rats (Brown and Rajan, 1986) maduramicin is mainly excreted in the faeces. The main metabolites resulted from O-demethylation of the methoxy groups. However, the site of O-demethylation was species-dependent. Metabolites of alpha-maduramicin were isolated and purified from turkey excreta. The major metabolic pathways for alpha-maduramicin in the turkey were O-demethylation in at least one of the methoxy groups in either the A- or G-ring followed by hydroxylation at an undefined position. Conjugation with glucuronic acid was identified as a minor route (Stout *et al.*, 1991).

Similar results in turkeys were reported by SCAN (EC, 1997). Maduramicin was labelled with <sup>14</sup>C at seven positions in the molecule. No balance study was performed. However, biliary excretion represented 22.7% of the administered dose, indicating considerable absorption. An examination of the nature of the excreted metabolites was carried out which showed parent maduramicin was the major compound (23.8%). The other identified metabolites corresponded to the double O-demethylation at C-44 and C-45 (G-ring) (17.9%), mono-O-demethylation at the same G-ring (4.2%), double O-demethylation at A-ring and G-ring (3.3%), O-demethylation at G-ring and hydroxylation at an undefined position (3.3%). A great number of very minor metabolites corresponding to more polar compounds were not identified.

Following a 7-day repeated administration to turkeys of <sup>14</sup>C-labelled maduramicin ammonium (7 mg/kg) the mean tissue residue levels measured at zero withdrawal time were 164, 137, 61, <25 µg maduramicin equivalents/kg (LOD) in fat, liver, skin/fat and muscle, respectively. After 1-day withdrawal all the tissues except the liver were <25 µg maduramicin equivalents/kg, and after 3-days the radioactivity was not detected in any of the tissues. More than 93.5% of the residual radioactivity in the fat was extractable of which 92.5% was parent maduramicin. The values for the liver were >89.0% and 51.1% respectively, and 14.2% (32

µg/kg) was tentatively identified as beta-maduramicin. A more polar metabolite (11.2%) was not identified, as well as several very minor metabolites all below 10% of the total radioactivity (EC, 1997).

Another study carried out in rearing conditions, i.e. unlabelled maduramicin for 100 - 107 days, confirmed the rapid elimination of the residual maduramicin (High performance thin layer chromatography and fluorescence detection at 25 µg/kg level); zero-day for the muscle, 1-day for the liver and 3-day for the skin/fat (EC, 1997).

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

No kinetic studies were available for non-target animal species.

## **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 intoxication and the limited number of tolerance studies and toxicological investigations in domestic animals show significant species differences in the susceptibility to maduramicin and a small margin of safety between the maximum authorised level for target animals and the minimum toxic dose in non-target animal species. Severe and even lethal intoxication and growth depression were observed particularly in cattle, sheep, chicken and rabbit.

In conclusion, accidental ingestion of feed for chickens for fattening containing the maximum authorised level of 5 mg maduramicin/kg feed pose a health risk for several non-target animal species including rabbits, cattle and sheep.

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

No quantitative data on maduramicin levels in feed for non-target animals are available.

Adverse effects of maduramicin have been observed in several non-target animal species (bodyweight gain and feed intake) at doses of 0.075, 0.15, 0.2 and 0.15 mg/kg b.w, per day for rabbits, horses, pigs and cattle, respectively. At higher doses congestive heart failure is observed.

Rates of cross-contamination of 2, 5 or 10% of the maximum authorised level of 5 mg maduramicin/kg feed for target animals, would result in concentrations of 0.1, 0.25 or 0.5

mg/kg in feeds given to non-target animals. A 10% cross-contamination and an average feed intake of 50 g/kg b.w. per day, applicable to most monogastric animal species, would lead to an intake of 0.025 mg/kg b.w., which is below the overall NOEL of 0.16 mg/kg b.w. per day derived from a two-year rat study. For rabbits, the most sensitive species, the LOAEL of 0.075 mg/kg b.w. per day is below this estimated intake. Hence it can be concluded that adverse health effects are unlikely to occur in non-target animal species as a result of cross-contamination of feed up to 10% of the maximum authorised level.

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

Residues of maduramicin in food were reported as non-compliant only on very few occasions. The concentrations found were not given and therefore it was not possible to use the data to estimate the exposure of maduramicin from eggs and animal tissues.

Because no kinetic studies in non-target animal species were available, the exposure of consumers to maduramicin as a result of consumption of foods from non-target animal species cannot be derived from such kinetic studies. Therefore, the CONTAM Panel used the data from chickens for fattening to estimate human exposure to maduramicin.

The kinetic data from chickens for fattening and turkeys show that residues occur predominantly in liver tissues but can also be found in skin/fat and muscle. By linear extrapolation from the chicken study a concentration of 3, 8 and 16 µg maduramicin/kg liver would be anticipated in chickens fed cross-contaminated diet at levels of 2, 5 and 10% (0.1, 0.3 and 0.5 mg/kg feed) of the maximum authorised level, respectively. The corresponding values for muscle are 0.4, 1 and 2 µg/kg. These concentrations represent the parent compound.

### **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**

No data were available to estimate the amount of maduramicin residues that might appear in eggs, milk or in meat and offal from non-target animal species. Hence, consumer exposure was estimated by extrapolating kinetic data from chickens for fattening to a diet cross-contaminated with 10% maduramicin of the maximum authorised level (i.e. up to 0.5 mg/kg feed). The calculated residues in the most relevant tissues, liver and muscle, at zero withdrawal time were 16 and 2 µg maduramicin/kg, respectively, and residues in kidney and skin/fat were negligible. Daily human consumption of 100 g of liver and 300 g of muscle<sup>17</sup>

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<sup>17</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)

would lead to an exposure of approximately 1.6 and 0.6 µg from liver and muscle, respectively, corresponding to a total of 2.2 µg/person and 0.037 µg/kg b.w. for a 60 kg person. This estimate corresponds to 3.7% of the ADI of 1 µg/kg b.w derived by the SCAN. Even though the kinetic behaviour and tissue deposition can differ between chickens for fattening and non-target animal species, an exceedance of the ADI is unlikely to occur. In addition, it was recognised that consumer exposure to residues of maduramicin resulting from cross-contamination of feed is likely to be infrequent.

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

## CONCLUSIONS

- Maduramicin exerts signs of toxicity typical of ionophoric compounds including cardiovascular effects and skeletal muscle lesions. Intoxications can be fatal and have been reported in non-target species (rabbits, cattle and sheep) at the maximum authorised level in feed for chickens for fattening and turkeys (5 mg/kg).
- Cross-contamination of feed for non-target animal species up to 10% (0.5 mg/kg feed) of the maximum authorised level of maduramicin is unlikely to represent a significant risk for non-target animal species. This concentration corresponds to a dose of approximately 0.025 mg/kg b.w. per day and is below the overall no-observed-effect-level (NOEL) of 0.16 mg/kg b.w. (based on a two year oral feeding study in rats). It is also below the LOAEL of 0.075 mg/kg b.w. (based on weight gain and feed intake) for rabbits, the most sensitive specie.
- No kinetic studies in non-target animal species were available. Therefore, the exposure of consumers to maduramicin as a result of consumption of foods was estimated from residues of maduramicin in the liver and muscle of chickens for fattening that were given maduramicin in their feed at 5 mg/kg at zero withdrawal time, extrapolated to a concentration of 0.5 mg/kg feed.
- 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 1 µg/kg b.w. established by the SCAN. Even though kinetic and tissue deposition can differ between chickens for fattening and non-target animal species, an exceedance of the ADI is unlikely to occur. The CONTAM Panel concluded that the very limited data provided no indication of an appreciable risk to consumers' health from the ingestion of

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and 100 g eggs (and 1500 g milk). Values for mammals are given in parenthesis when they differ from bird values

maduramicin residues in products from animals exposed to feed cross-contaminated up to a hypothetical level of 10% of the maximum authorised level.

## **RECOMMENDATIONS**

- Sensitive analytical methods that have become available for the detection of maduramicin in animal products should be validated also for feed concentrations below the maximum authorised level, to assess their applicability in the control of undesirable cross-contamination of feed batches during the production process.
- In countries where maduramicin is used, regular surveys for residues in animal products are recommended in order to evaluate human exposure to maduramicin.

## **REFERENCES**

- Alpharma, Gromax Technical Manual. Alpharma Animal Health Division. [www.alpharma.com.br/pg/pdf/mt-gromax.pdf](http://www.alpharma.com.br/pg/pdf/mt-gromax.pdf)
- Badiola, J.J., Fernandez de Luco, D., Perez, V., Vargas, M.A., Lujan, L. and Garcia-Marin, J.F. 1994. Maduramicin and tiamulin compatibility in broiler chickens. *Avian Pathology* 23: 3-17.
- Bastianello, S.S., Fourie, N., Prozesky, L., Nel, P.W. and Kellermann, T.S. 1995. Cardiomyopathy of ruminants induced by the litter of poultry fed on rations containing the ionophore antibiotic, maduramicin. II. Macropathology and histopathology. *Onderstepoort J Vet Res* 62: 5-18.
- 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.
- Brown, M.A. and Rajan, S. 1986. Maduramicin: rat metabolism of a highly potent polyether anticoccidial examined by Carbon-13 NMR. *J Agric Food Chem* 34: 470-472.
- De Jong, J., Stoisser, B., Wagner, K., Tomassen, M., Driessen, J., Hofmann, P. and Putzka, H.A. 2004. Determination of maduramicin in feedingstuffs and premixtures by liquid chromatography: development, validation, and interlaboratory study. *J AOAC Int* 87 (5): 1033-1041.
- 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.

- EC (European Commission), 1988. The use of maduramicin-ammonium in feedingstuffs for fattening chicken. EUR 12824 EN. Reports of the Scientific Committee for Animal Nutrition, 7th series, Commission of the European Communities, Agriculture, pages 1-8.  
[http://ec.europa.eu/food/fs/sc/oldcomm6/antibiotics/24\\_en.pdf](http://ec.europa.eu/food/fs/sc/oldcomm6/antibiotics/24_en.pdf)
- EC (European Commission), 1997. Report of the Scientific Committee for Animal Nutrition on the use of maduramicin ammonium in feedingstuffs for turkey. Opinion expressed, 25 September 1997; Text consolidated, 24 October 1997.  
[http://ec.europa.eu/food/fs/sc/oldcomm6/antibiotics/53\\_en.pdf](http://ec.europa.eu/food/fs/sc/oldcomm6/antibiotics/53_en.pdf)
- Fourie, N., Bastianello, S.S., Prozesky, L., Nel, P.W. and Kellerman, T.S. 1991. Cardiomyopathy of ruminants induced by the litter of poultry fed on rations containing the ionophore antibiotic, maduramicin. I. Epidemiology, clinical signs and clinical pathology. *Onderstepoort J Vet Res* 58: 291-296.
- 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>
- ISO (International Standards Organization), 1997. Capability of detection — Part 1: Terms and definitions, Part 2: Methodology in the linear calibration case Part 2: Methodology in the linear calibration case. 11843.
- IUPAC (International Union of Pure and Applied Chemistry), 1995. Protocol for the design, conduct and interpretation of method-performance studies. *Pure Applied Chem* 67: 331.
- Jestoi, M., Rokka, M. and Peltonen, K. 2007. An integrated sample preparation to determine coccidiostats and emerging Fusarium-mycotoxins in various poultry tissues with LC-MS/MS. *Mol Nutr Food Res* 51: 625-637.
- Kennedy, D.G., Hughes, P.J. and Blanchflower, W.J. 1996. Ionophore residues in eggs in Northern Ireland: incidence and cause. *Food Add Cont* 15: 535-541.
- Kennedy, D.G., Blanchflower, W.J. and O'Dornan, B.C. 1997. Development of an ELISA for maduramicin and determination of the depletion kinetics of maduramicin residues in poultry. *Food Addit Contam* 14: 27-33.
- Kennedy, D.G., Smyth, W.G., Hewitt, S.A. and Mc Evoy, J.D. 1998. Monensin carry-over into unmedicated broiler feeds. *Analyst* 123 (12): 2529-2533.
- Mc Evoy, 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.
- Noser, J., Wenk, P., Sutter, A. and Schneider, P. 2006. Kokzidiostatika in Eiern, Geflügelfleisch und Futtermitteln: Eine Stichproben-Kontrolle über den Nordwestschweizer Markt. *Mitt Lebensm Hyg* 97: 107-120.
- Sanford, S.E. and McNaughton, C. 1991. Ionophore (maduramicin) toxicity in pigs. *Can Vet J* 32 (9): 567.
- Sharma, N., Bhalla, A., Varma, S., Jain, S. and Singh, S. 2005. Toxicity of maduramicin. *Emergency Medicine Journal* 22(12): 880-882.
- Shen, J., Qian, C., Jiang, H. and Yang, H. 2001. Development of an enzyme-linked immunosorbent assay for the determination of maduramicin in broiler chicken tissues. *J Agric Food Chem* 49: 2697-2701.

- Schlosberg, A., Perl, S., Harmelin, A., Hanji, V., Bellaiche, M., Bogin, E., Cohen, R., Markusfeld-Nir, O., Shpigel, N., Eisenberg, Z., Furman, M., Brosh, A., Holzer, Z. and Aharoni, Y. 1997. Acute maduramicin toxicity in calves. *Vet Rec* 21: 643-646.
- Singh, T. and Gupta, R.P. 2003. Clinico-haematological and mineral studies on experimental maduramicin toxicity in chickens. *Vet Parasitol* 116(4): 345-53.
- Stout, S.J., Wu, J., Dacunha, A.R., King, K.G. and Lee A.H. 1991. Maduramicin alpha - characterization of C-14 derived residues in turkey excreta. *Journal of Agricultural and Food Chemistry* 39: 386-391.
- 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.

## **DOCUMENTATION PROVIDED TO EFSA**

- Alpharma, Animal Health Division, 2006. Maduramicin ammonium. Tolerance data in non-target species and residue data in non-target species. Confidential reports.
- 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 authorised 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.

## **Occurrence data**

- Belgium. AFSCA, The Food Agency.
- Czech Republic. Central Institute for Supervising and Testing in Agriculture.
- European Commission, DG SANCO.