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Cross-contamination of non-target feedingstuffs by salinomycin authorised for use as a feed additive[1]

Scientific Opinion of the Panel on Contaminants in the Food Chain

Question N° EFSA-Q-2005-220C

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SUMMARY

Salinomycin sodium is a polyether carboxylic ionophore agent that is authorised according to Regulation No (EC) 1831/2003 as a coccidiostat for use in chickens for fattening with a maximum content of the active ingredient in feed of 70 mg/kg and a withdrawal period of one day, for chickens reared for laying (up to 12 weeks of age) with a maximum content of 50 mg/kg and no withdrawal period, and for rabbits for fattening with a maximum concentration in feed of 25 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 subsequent feed batches. This cross-contamination may result in the exposure of non-target animal species, and hence the potential health risks for non-target animal species as well as the potential residue deposition in foods derived from these non-target animal species have been evaluated.

[1] For citation purposes: Opinion of the Scientific Panel on Contaminants in the Food chain on a request from the European Commission on cross-contamination of non-target feedingstuffs by salinomycin authorised for use as a feed additive, *The EFSA Journal* (2008)591, 1-38.

Signs of intoxication, including cardiovascular effects (raised blood pressure and myocardial degeneration), anorexia, weakness, ataxia, paralysis have been reported in various non-target animal species. These signs correlate with the mode of action of ionophores and occur also in target animal species at dose levels exceeding the maximum authorised level. Particularly sensitive to salinomycin are turkeys, pre-ruminant calves and cattle, horses, cats and dogs, whereas pigs are less sensitive. In dogs, signs of neurotoxicity have been observed in experimental studies. Toxicity has been described in some non-target animal species at salinomycin concentrations in feed below the maximum level authorised for chickens for fattening, hence reflecting the significant species difference, and the small margin of safety of sodium salinomycin used as a coccidiostat. The Panel on Contaminants in the Food Chain (CONTAM) concluded that ingestion of the maximum authorised level of salinomycin in poultry feed of 70 mg/kg feed) may cause intoxications and constitute a health risk for several non-target animal species.

Cross-contamination of feed for non-target animal species at a hypothetical level of 10% (7 mg/kg feed) of the maximum authorised level of salinomycin in feed for target animal species could result in an intake of 0.35 mg salinomycin/kg b.w. per day. This level is below the no observed adverse effect level (NOAEL) of 0.5 mg/kg b.w. identified by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) from a one-year feeding study in dogs, which are known to be among the most sensitive animal species. However, horses have been shown to be much more sensitive to acute oral exposure to salinomycin than dogs, with lethal doses being as low as 0.12-0.25 mg/kg b.w. (corresponding to feed cross-contaminated at levels of 3 to 6%). The CONTAM Panel concluded that adverse health effects in horses from intake of salinomycin from cross-contaminated feed are likely to occur, even at a level of 2% cross-contamination. Toxic effects are not expected for other non-target animal species.

Kinetic studies in various animal species showed that salinomycin is rapidly absorbed, extensively metabolised and cleared entirely from the body within a few days. No data on possible carry-over into milk are available. The potential human exposure was calculated using kinetic data and the results from the residue surveillance studies conducted by the Member States. The estimated dietary exposure of consumers was well below the ADI of 5 μg/kg b.w. as established by the FEEDAP Panel.

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

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

[2] 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)

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[3]

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[4] 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

[3] OJ L 35, 8.2.2005, p. 1
[4] 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.

Salinomycin sodium has most recently been assessed by the FEEDAP Panel (EFSA, 2004a,b,c, 2005, 2006), and salinomycin based products has been authorised for use as feed additives in accordance with the provisions of Council Directive No (EC) 70/524 (see Table 1).

Regulation No (EC) 1831/2003[5] of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition is applicable since 19 October 2004 and repeals Directive No (EC) 70/524 with effect from that date.

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

Cocciodiostat (active substance)	**Species or category of animals for which the use of coccidiostats is authorised (target animal)**	**Authorised maximum content of active substance in complete feed**
Salinomycin sodium	Chickens for fattening Chickens reared for laying (max 12 weeks) Rabbits for fattening	70 mg/kg (Salinomax™, Sacox™) 50 mg/kg (Sacox™) 25 mg/kg (Sacox™)

4. Unavoidable cross-contamination (under practical conditions)

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

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

5. Tolerances

The possibility to set tolerances for these in practice unavoidable residues of coccidiostats in feedingstuffs for non-target animal species should be considered in the frame of Directive No

[5] OJ L 268, 18.10.2003, p. 29–43

(EC) 2002/32[6] of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

Such tolerances in feedingstuffs for non-target animal species could be set following the ALARA principle (As Low As Reasonably Achievable) taking into account good manufacturing practices. According to information received from professional organisations, 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 animal species 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 animal species 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 salinomycin sodium authorised as feed additive into feeds for non-target animal species.

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

The EFSA is requested to provide an opinion whereby

- the animal health risk for non-target animal species (food producing farm animals) will be assessed,
- the adverse effects as a consequence of cross-contamination of salinomycin sodium into feed for non-target animal species,
- on the basis of the available information, an estimate of the level of residues present in food of animal origin from non-target animal species as the consequence of cross-contamination is performed,
- the possible risks for human health as the consequence of the presence of such residues in food of animal origin (eggs, milk, meat, edible offal) from non-target animal species are assessed.

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

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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 Directive No (EC) 70/524 and Regulation No (EC) 1831/2003 that repeals Directive No (EC) 70/524 (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[7] or the JECFA[8]) 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.

[7] The Committee for Medicinal Products for Veterinary Use of the European Medicines Agency

[8] 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).

MRL values: Maximum residue limits. The CVMP applied Regulation No (EC) 1055/2006[9] amending the Annexes I and III of Regulation No (EC) 2377/90[10] 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.

Residues of coccidiostats in edible tissues, milk and eggs: According to Directive No (EC) 96/23[11] 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 ^{14}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

Salinomycin is a polyether carboxylic acid ionophore. It is isolated from *Streptomyces albus*, and exerts both antimicrobial and anticoccidial effects. The structural formula of salinomycin is presented in Figure 1; it is used as the sodium salt (CAS Number 55721-31-8) and salinomycin free acid (CAS Number 53003-10-4).

[9] OJ L 192, 13.7.2006, p. 3–5
[10] OJ L 224, 18.8.1990, p. 1–8
[11] OJ L 125, 23.5.1996, p. 10–32

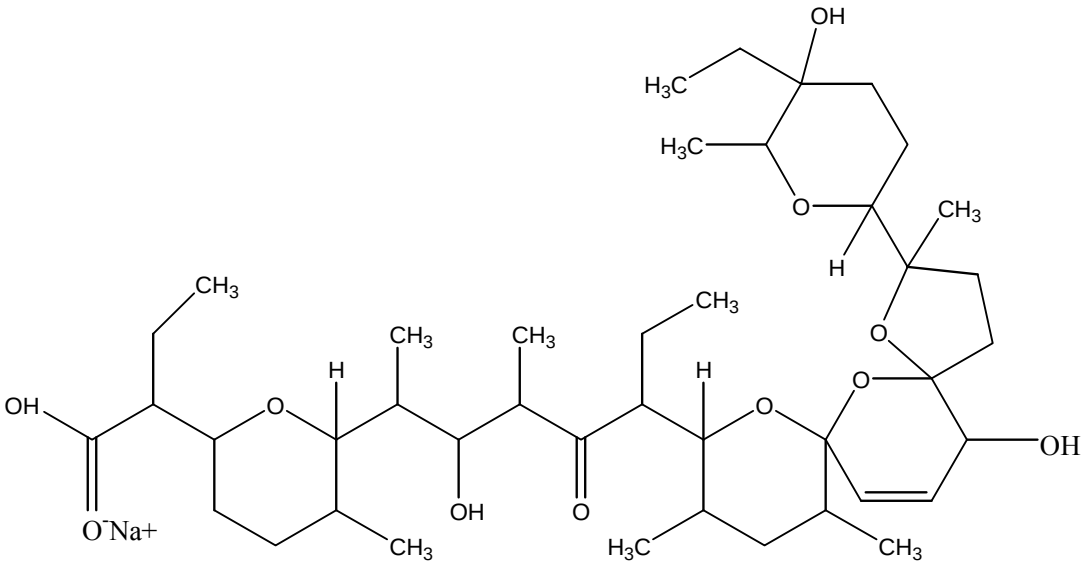

Figure 1: Chemical structure of salinomycin sodium.

The IUPAC name of salinomycin sodium is ethyl-6-[5-{2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyrano-2-yl)-15-hydroxy-2,10,12-trimethyl-1,6,8-trioxadispiro [4,1,5,3] pentadec-13-en-9-yl} 2-hydroxy-1,3-dimethyl-4-oxoheptyl] tetrahydroxy-5-methyl-2H pyran-2-acetic acid, sodium The molecular weight is 773 and the molecular formula is $C_{42}H_{69}O_{11}Na$. The water solubility of salinomycin sodium is approximately 17 mg/L, the log octanol/water partition coefficient (Kow) is 5.15 and the vapour pressure negligible (EFSA, 2004a).

As summarised in the background chapter, salinomycin sodium is authorised as a coccidiostat to be used in feeds for chickens for fattening at a minimum-maximum concentration of 50-70 mg/kg feed and a withdrawal period of one day (Regulation No (EC) 496/2007[12] and 500/2007[13]). Two individual products (Salinomax® and Sacox®) are authorised. One of these (Sacox®) is also authorised for chickens reared for laying (up to a maximal age of 12 weeks) at 50 mg/kg feed without a withdrawal period (Regulation No (EC) 1852/2003[14]), and in rabbits for fattening at 20-25 mg/kg feed, and a withdrawal period of five days (Regulation No (EC) 937/2001[15]). Recently, the FEEDAP Panel has assessed Kokcisan® for use in feed for chickens for fattening at a minimum-maximum concentration of 60-70 mg/kg.

1.1. Biological activity of salinomycin

Anticoccidial activity

[12] OJ L 117 5.5.2007, p.9-10
[13] OJ L 118, 8.5.2007, p. 3-4
[14] OJ L 271, 22.10.2003, p.13
[15] OJ L 130, 12.5.2001, p. 25–32

Salinomycin, like other polyether ionophores, is effective against sporozoites, and early and late asexual stages of coccidia in the intestines of chickens. The biological activity is based on the ability of ionophores to form lipid soluble and dynamically reversible complexes with ions (preferably the alkali ions K^+ and Na^+). Salinomycin encloses the cation in a hollow ball, in the centre of which the cation is fixed and immobilised. It functions as a carrier of ions, mediating an electrically-neutral exchange of cations across the membranes. The resultant changes in trans membrane ion gradients and electrical potentials often produce profound effects on cellular function and metabolism that can lead to the death of the coccidia (EFSA, 2004a,b,c).

Antibacterial activity

Salinomycin shows a selective antibacterial activity in a concentration range of 0.5 to 16 mg/L against many Gram-positive bacterial species, whereas *Enterobacteriaceae* are resistant. The minimum inhibitory concentration (MIC) of salinomycin for common intestinal bacterial species such as *Enterococcus faecalis, E. faecium, Staphylococcus spp.* and *Clostridium perfringens* range between 0.5 and 16 mg/L. Inhibitory concentrations of salinomycin for susceptible bacterial strains are thus lower than the concentration in supplemented feed. Increased shedding of *Salmonell*a spp., Campylobacter and Clostridium was not shown to occur, neither under experimental nor under practical conditions (EFSA, 2004a).

Enterococci may develop resistance to salinomycin. In the European Union the development of harmonized antimicrobial resistance and surveillance programs in animals and animal-derived foods have been initiated in the last ten years. In the monitoring programs resistance to salinomycin in chickens for fattening isolates of *Enterococcus faecalis* and *Enterococcus faecium* was monitored and reported. For example, in Denmark, salinomycin susceptibility has been monitored since 1997 in *Enterococcus faecium* and *E. faecalis* strains collected from poultry, pigs and calves. The salinomycin MIC90 of *Enterococcus faecalis* increased between 1998 and 2002 in poultry from 0.5 to 8 µg/mL. This reflects a shift in the MIC distribution of *Enterococcus* isolates of poultry origin. In Belgium resistance against salinomycin was frequent among poultry strains of *E. faecalis* and *E. faecium*. In the Netherlands, no salinomycin resistance was reported in *E. faecium* poultry isolates (EFSA, 2004a).

Although the selection of salinomycin resistant enterococci has been demonstrated, resistance to salinomycin is not associated with cross-resistance to antibiotics used for therapy in human or veterinary medicine. Salinomycin is not classified as a critically important antibiotic for human medicine by the WHO expert Panel, but has been classified by Office Internationale des Epizooties (OIE, World Organization for Animal Health) (OIE, 2006) as a highly important antibiotic in the control of coccidiosis in farm animals.

The Scientific Committee for Animal Nutrition (SCAN) did not evaluate the effect of salinomycin on the bacterial populations that make up the human gut flora. The FEEDAP considered the antimicrobial activity of salinomycin but did not establish an antimicrobial ADI. The CONTAM Panel noted that the FEEDAP Panel did consider this issue in the

opinions on coccidiostats for target animals, and hence this issue was not included in the opinions of the CONTAM Panel on coccidiostats in non-target animal species.

1.2. Previous evaluations of salinomycin

Salinomycin toxicity has been assessed by the SCAN (EC, 1992) and more recently the FEEDAP Panel (EFSA, 2004a,b,c, 2005, 2006) in the frame of the authorisation for the use of various feed additives containing salinomycin (the original data were not available to the CONTAM Panel). According to these reports the oral median lethal dose (LD_{50} values) were found to vary from 21 mg/kg in rabbits, 46-124 mg/kg for rats to 50-148 mg/kg for mice, the female being more sensitive. The SCAN identified a NOEL of 0.25 mg/kg b.w. per day from a teratogenicity study in rabbits in which there were increased numbers of resorptions at oral doses of 0.63 and 1.6 mg/kg b.w. per day. Reduced litter size was seen in one field trial in which rabbits were fed diet containing 25 mg salinomycin/kg feed (corresponding to 0.75 mg/kg b.w. per day), but this effect was not seen in other field trials in which rabbits were fed 20-75 mg salinomycin/kg feed. On the basis of the NOEL from the rabbit teratology study, the SCAN established an ADI of 0.0025 mg/kg b.w. per day (EC, 1992). More recently, the FEEDAP Panel based its assessment of the salinomycin-based products Sacox and Salinomax on the results of a one-year oral study in dogs in which the NOAEL was 0.5 mg/kg b.w. per day. The endpoints on which the NOAEL was based were neurotoxic effects characterised by myelin loss, primary axonal degeneration and Wallerian-like degeneration. The ADI was then derived by applying a 100-fold uncertainty factor to give a provisional ADI of 0.005 mg/kg b.w. per day (EFSA, 2004a,b; 2005). Oral pharmacological studies in dogs showing an increase in heart rate indicated a NOAEL of 0.625 mg/kg b.w. Other toxicity studies for sodium salinomycin were also analysed by the FEEDAP panel and included a large number of oral repeated dosing toxicity studies in mice, rats and rabbits, a fertility study in rats, a three-generation reproduction study in rats, developmental toxicity studies in mice, rats and rabbits (EFSA, 2004a,b,c). The report of the rabbit teratology study that the SCAN had used to identify its ADI was not available to the FEEDAP and the Opinion of SCAN (EC, 1992) was insufficiently detailed to allow FEEDAP to evaluate this study. However, other studies of the effects of salinomycin on reproduction and development in rabbits were available and their results indicated that these endpoints were not critical to the setting of the ADI for salinomycin. Studies of *in vitro* MIC values of 109 strains of bacteria were provided and showed that they were mostly insensitive to salinomycin. The microbiological data were not used in the calculation of the ADI, but would not be expected to cause the ADI to be set at a lower value. One of the *in vitro* genotoxicity studies gave a positive result for production of chromosomal aberrations. However, *in vitro* tests for bacterial mutations and gene mutations in mammalian cells all gave negative results. *In vivo* mutation assays i.e. sex-linked recessive lethal mutation assay in fruit flies, a bone marrow micronucleus assay in male and female CD-1 and NMI mice and a DNA-repair assay in rat liver unscheduled DNA synthesis also gave negative results and it was concluded that salinomycin was not genotoxic.

Carcinogenicity studies in mice and in rats were uniformly negative (EFSA, 2004a, 2006). A lack of data on chronic toxicity was noted for one product containing salinomycin (Kokcisan 120G), leading the FEEDAP Panel to require further studies (EFSA, 2006) which have been performed recently. These 2 year long-term studies in rats with Kokcisan 120G have shown that salinomycin is not carcinogenic and a LOEL of 1.5 mg/kg b.w. per day, based on haematological and blood chemistry effects, could be identified (EFSA, 2007). This LOEL is three fold larger than the lowest NOAEL for short terms studies (0.5 mg/kg b.w. per day) and the FEEDAP Panel retained the ADI of 5 µg/kg b.w. per day (0.3 mg/kg b.w. for a 60 kg person). This ADI has replaced the earlier ADI that was set by the SCAN.

Maximum residue limits (MRLs) of 5 µg/kg for the chicken liver, kidney and muscle and 15 µg/kg for chicken skin/fat were established for salinomycin by regulation No (EC) 496/2007[16] and 500/2007[17].

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 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

[16] OJ L 117, 5.5.2007, p.9
[17] OJ L 118, 8.5.2007, p. 3–4

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, nicarbazin 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 salinomycin-based feed additive products

Salinomycin is very stable under UV light, heat, and oxidative and alkaline conditions, but very unstable under acidic conditions. Salinomycin sodium is stable under normal storage conditions for 6 to 24 months, supporting a two-year shelf life. Salinomycin sodium was stable during feed processing. Studies with premixes and with complete feeds demonstrated a satisfactory stability of salinomycin sodium during 6 and 4 months, respectively (25ºC/50% relative humidity; 40ºC/75% relative humidity) (EFSA, 2004a,c).

Particle size/dusting potential (Stauber-Heubach) of salinomycin sodium:
Corresponding data for Bio-Cox 120 G (now re-named Salinomax 120G) and Sacox 120 microGranulate are shown in Table 2.

Table 2. Data on particle size and dusting potential (Stauber-Heubach) of approved salinomycin containing products (12% concentration).

Brand name	Particle size	Dusting potential
Bio-Cox 120 G (EFSA, 2004c)	Granulated 100% > 50 µm 0.55% 50-150 µm 12.6% 150-250 µm 54.5% 250-425 µm 32.3% 425-850 µm 0.05% > 850 µm	5-8 mg active substance/m³ Concentration of additive in the dust is not specified
Sacox 120 microGranulate (EFSA, 2004a)	Granulated 3% < 5 µm 9.7% 0.5-45 µm 21.5% < 125 µm 50% 125-315 µm 47.3% < 224 µm 98.8% < 500 µm	0.45 g/m³ air Dust contains 178 mg/g active substance instead of 120 mg/g, e.g. enrichment of the active substance in the dust

Dusting behaviour of feed additives, which were tested at IFF Research Institute of Technology, Germany, during the last years, indicates that 50% of all products had a dusting potential under 0.500 g/m³ and 20% under 0.200 g/m³. The tested Sacox 120 microGranulate product is within this range (Feil, 2007, personal communication).

Data for Sacox 120 microGranulate showed an enrichment of the active substance in the dust (178 mg/g instead of 120 mg/g) (EFSA, 2004a). No survey has been conducted to verify these enrichments under practical conditions.

2. Methods of analysis for salinomycin

2.1. Analysis of salinomycin in premixes and animal feeds

In the process of approval of a feed additive containing salinomycin, the applicants presented analytical methods for quantification of salinomycin in supplemented feed. Moreover, several other methods are in use in Member States.

For Sacox, the method is based on derivatisation using vanillin with limit of detection (LOD) and limit of quantification (LOQ) values of 5 and 15 mg/kg feed, respectively (EFSA, 2004a).

For Bio-cox (Salinomax), the method is based on derivatisation using p(dimethylamino)benzaldehyde with a LOQ value of 0.2 mg/kg feed (EFSA, 2004c). The level of salinomycin used in feed varies from 20 to 70 mg/kg in complete feeds depending on the animal species. The risk of cross-contamination between batches necessitates analytical methods with sensitivity within the region of 200 µg/kg complete feed which equals 1% contamination.

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

An analytical procedure for the detection of salinomycin in poultry feeds used methanol extraction and direct derivatisation with 2,4-dinitrophenylhydrazine. The derivatisation mixture was analysed with ultraviolet (UV) detection at 305/392 nm. The recoveries of the salinomycin from spiked samples were 85-100% with a relative standard deviation (RSD) of 4-10% in a concentration range of 50-150 mg/kg. The LOD was 20 mg/kg (Dusi and Gamba, 1999).

A liquid chromatographic method for salinomycin in animal feed used hexane-ethyl acetate extraction and UV detection after post-column reaction with vanillin. The method was applied to poultry and pig feeds with levels of 3-100 mg salinomycin/kg. Recoveries for the LC method ranged from 92.1 to 103% with an average recovery of 98.1% and a coefficient of variation of 3.65% (Lapointe and Cohen, 1988).

Only one of the methods reviewed (LC-MS), had a reported detection capability close to the µg/kg level in complete feed, and a good recovery of the analyte (Turnipseed *et al.,* 2001) and would therefore be able to quantify salinomycin in cross-contaminated feeds for non-target animal species.

2.2. Analysis of salinomycin residues in animal products

Analytical methods were described and validated by applicants for detection of salinomycin in chicken tissues with LODs between 1 µg/kg and 5 µg/kg (EFSA, 2004a,b,c).

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), salinomycin residues are screened in different matrices such as eggs, poultry meat and bovine, pigs, rabbit, sheep and goats by 20 member states. The Member States used different methods such as enzyme immunoassay (EIA), high performance liquid chromatography (HPLC), high

performance thin layer chromatography (HPTLC) and HPLC-MS or HPLC-MS/MS. The decision limits ranged between 1 and 200 µg salinomycin/kg tissue.

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

2.2.1. Screening methods

A screening method based on rapid time-resolved fluoro-immunoassay (TR-FIA) was described by Peippo *et al.* (2004) for the screening of salinomycin in muscle and eggs. The LOD for salinomycin was 0.56 and 0.28 µg/kg for muscle and eggs, respectively.

Heller and Nochetto (2004) described a screening method based on Ion-Trap LC-MS/MS for several non-polar residues in eggs. The LOD of this method was 1 µg/kg for salinomycin in eggs.

2.2.2. Quantitative and confirmatory methods

Hormazabal and Yndestad (2000) presented a multi-residue method based on LC-MS for analysis of salinomycin in eggs, fat, liver and muscle. The limits of detection for salinomycin were 5, 3, 5 and 3 µg/kg product, respectively.

Another multi-residue method based on LC-MS/MS was described by Rosen (2001) for the screening of salinomycin residue in eggs and chicken liver. The LOD was 0.04 µg/kg.

A multi-residue quantitative method based on LC-MS/MS was also described by Mortier *et al.* (2005a) for analysis of four coccidiostats, including salinomycin, in eggs. For salinomycin, the decision limit[18] (CCα) was 1 µg/kg.

Finally, an LC-MS/MS multi-residue method was described by Rokka and Peltonen (2006) for the quantitative detection of four coccidiostats (lasalocid, monensin, salinomycin and narasin) in eggs and chicken meat. With this method, the decision limits (CCα) for salinomycin were 0.9 and 2.5 µg/kg in eggs and muscle, respectively.

[18]Definitions of limit of detection (LOD), limit of quantification (LOQ), decision limit (CC α) and detection capability (CCβ): Commission decision 2002/657of 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. CCα means the limit at and above which it can be concluded with an error probability of α that a sample is non-compliant. CCβ means the smallest content of the substance that may be detected, identified and/or quantified in a sample with an error probability of β. CCα 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).

3. Occurrence of salinomycin

3.1. Occurrence of salinomycin 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. No residues of salinomycin were found with a LOD of 0.5 mg/kg (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 animal species which was prepared following the production of feed for target animal species and a cleaning procedure. Thirteen positive samples were found. They contained salinomycin at levels between 0.07 and 2.95 mg/kg feed (data provided to EFSA).

Information from the Rapid Alert System for Food and Feed (RASFF)[19] between April 2002 and April 2006 showed seven incidents in which salinomycin was found in feed materials for non-target animal species (data provided by the European Commission). The amounts detected which could be explained by cross-contamination of feed were 1.0, 1.9 and 6.1 mg/kg. In addition, there were two cases with levels of 79 and 135 mg salinomycin/kg feed in complete feed for turkeys and 722 and 830 mg/kg in mineral feed for dairy cattle, these high levels can not be a result of cross-contamination, but are most likely accidental contaminations (data provided by the European Commission).

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

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

Eggs, muscle and liver from different food-producing animal species are analysed for residues of coccidiostats by the Member States according to requirements in Directive No (EC) 96/23 Combined results for salinomycin from 2004 and 2005 show 30 non-compliant samples from a total of 15151 analyses, 19 of them in eggs from a total of 3502 analyses, 7 from pig liver, 3 from poultry (of which at least 2 were from liver) and one from rabbit liver or muscle. A total

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

of 6902 liver samples were analysed. The non-compliant liver samples were all from target animals (pigs were target animals until 1 January 2006) and data obtained before 1 January 2006 will not be used in the exposure assessment for salinomycin residues in products of non-target animal species (data provided by the European Commission).

The results were very different in terms of detection limits 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 salinomycin/kg tissue as their non-compliant limit. In addition, no details are given of the concentrations of salinomycin residues that were found in the non-compliant samples. The LOD ranged between 0.03 and 100 μg/kg.

Belgium has provided individual data for 958 samples of muscle tissue from different animals and eggs that were analysed in 2005 and 2006. Three samples (25.7 μg/kg in sheep muscle, 54 and 141 μg/kg in eggs) contained concentrations above the Belgian non-compliant limit of 10 μg/kg and seven samples were between the LOD of 2 μg/kg and the Belgian non-compliant limit. Danish results for 776 samples show residues in 17 of 182 egg samples all between the LOD of 0.1 μg/kg and their non-compliant limit of 10 μg/kg (data provided to EFSA).

Targeted surveys of 7409 food samples collected in the United Kingdom (UK), or imported into the UK, over an eleven-year period of 1995-2005, showed seven samples containing residues of salinomycin (UK-VMD, 1995-2005). The foods tested were chicken eggs (2133 eggs tested); liver of chickens for fattening (1837 samples tested), hens (93), chickens (79), turkeys (459), ducks (34), quail (15), calves (238), cattle (53), pigs (128) and sheep (1765); meat from chickens (41), turkeys (70), rabbits (114), sheep (60) and deer (20); breaded turkey products (20); poultry burgers (40); pâtés from livers of chicken and pigs (40); baby foods based on chicken (100) and on eggs (70). LOQs for the analytical methods varied between 1 and 50 μg/kg. Salinomycin was present in two chicken egg samples at concentrations of 15 μg/kg and 20 μg/kg. Measurable levels of salinomycin in the liver were found at a concentration of 30 μg/kg in one sample from chickens for fattening, at 4 μg/kg in one sample of sheep liver, at 11, 15 and 20 μg/kg in three samples of pig liver. Salinomycin was not detected in any food samples taken in the period 1996-1999 nor in 2002 and 2004 (UK-VMD, 1995-2005).

In a survey of 320 egg samples, purchased in eight different European countries, eggs were analysed for the presence of nine different coccidiostats including salinomycin. Salinomycin was found in 50 samples and was the coccidiostat most frequently found, accounting for 44% of all positive samples with 88% of these positives having concentrations lower than 1 μg/kg. The highest concentration of salinomycin found was 63 μg/kg. The CCα was below 1 μg/kg (Mortier *et al.,* 2005b).

4. Toxicity of salinomycin

4.1 Mechanism of toxicity

Ionophores modify the permeability of biological membranes by forming lipid soluble, dynamically reversible cation complexes and these complexes transport cations across biological membranes. Each carboxylic ionophore species has its own characteristic inorganic ion selectivity pattern. Furthermore, ionophores also differ in molecular polarity, which affects their differential distribution in biological membranes.

4.2. Toxicity of salinomycin in target animal species

The toxicity of salinomycin in chickens of different age groups and rabbits has been assessed recently by the FEEDAP Panel (EFSA, 2004a,b,c; EC, 1992).

4.2.1 Chickens

Chickens (5 males and 5 females per group) received salinomycin sodium for 56 consecutive days at concentrations of 0, 60, 120 and 150 mg/kg feed, respectively. A reduction in feed consumption was observed at all salinomycin levels, but there were no clinical signs of toxicity. Birds treated at the highest concentration (150 mg/kg) showed a 25 g/day decrease in feed intake associated with considerable growth rate depression after 56 days (EFSA, 2004b).

4.2.2 Rabbits

In rabbits, concentrations of 20-25 mg/kg in complete feedingstuffs have been shown to be effective in protecting rabbits from coccidiosis. The 1992 the SCAN evaluation concluded that salinomycin was embryotoxic in rabbits and that these effects may occur at concentrations below 20 mg/kg of complete feed (EC, 1992). Hence, salinomycin has not been authorised for breeding animals.

In an old study provided to EFSA, rabbits were fed a diet containing salinomycin at levels of 0, 50, 75, 100 and 125 mg/kg complete feed and body weight gains were reduced at the two highest doses. The NOAEL was considered to be 75 mg/kg of diet corresponding to 5.6 mg/kg b.w. per day (data provided by industry).

4.3. Toxicity of salinomycin in non-target animal species

4.3.1. Turkeys

Case reports have described a high mortality (23 to 90%) in adult male and female turkey breeders when given feed containing salinomycin at levels from 24 to 37 mg/kg (Halvorson *et al.,* 1982). Other reports have shown lower mortality rates in turkeys (1.6-15.8%) at levels of 15-30 mg/kg feed (Stuart, 1983) and 21.7% at levels of 13-18 mg/kg feed (Andreasen and Schleifer, 1995). Clinical signs included reduced food intake, dyspnoea, drooping wings, reluctance to move, abnormal gait and reduced reproductive performance. The most common histopathological findings were skeletal muscle degenerations and necrosis. Mortality occurred within 5 to 12 hours after the development of clinical signs. Salinomycin has also been shown to become more toxic as the age of the turkeys increased (Potter *et al.,* 1986). A recent case report describes a mortality rate of 2.57% in turkeys fed with feed containing 60 mg/kg salinomycin sodium. The affected animals showed signs of dyspnea, drowsiness, sternal recumbency with legs extended posteriorly, inability to stand, stiffness and weakness. Histological examination showed severe fragmentation and necrosis of muscle fibres in most sections, and eosinophilic myocardial fibres undergoing fragmentation (Van Assen, 2006).

4.3.2. Horses

There have been worldwide reports on the toxicity of ionophores to horses for decades. Rollinson *et al.* (1987) observed six cases of accidental salinomycin poisoning in horses. The range of clinical signs included anorexia, colic, weakness and ataxia. More recently, Nicpon *et al.* (1997) reported accidental intoxication of 24 horses fed 2-3 kg feed containing 61 mg salinomycin sodium/kg corresponding to 0.12-0.25 mg/kg b.w. Only six horses survived. The most characteristic clinical sign appears to be the paralysis of the hind limbs.

One specific study was conducted with two horses (one gelding and one mare) to assess the effects of accidental exposure to salinomycin in the diet (data provided by industry). Salinomycin sodium was incorporated into a pig diet at concentrations of 60, 120 and 180 mg/kg and fed to the horses resulting in exposures of 0.15, 0.2 and 0.6 mg/kg b.w., respectively. No adverse effects were observed at the doses of 0.15 and 0.20 mg/kg b.w. but feeding time was prolonged at these doses to 5-7 hours. Approximately 50 hours after receiving the highest dose (0.6 mg/kg b.w.), one horse developed immobility and had increased heart and respiratory rates. *Post mortem* and histopathological investigations indicated cardiac insufficiency with stasis-induced hyperaemia and pulmonary oedema. Additional findings included fat accumulation in the liver and myocardium, and cardiac muscle degeneration, scar formation and destruction of skeletal musculature.

4.3.3. Ruminants

A single dose study evaluated the toxicity of salinomycin in four black fattening steers via nasogastric tube at doses of 8, 10 and 15 mg/kg b.w. Adverse effects were reported at 8 mg/kg within two to six hours such as cardiovascular disturbances, tremor and rejection of food. Symptoms persisted for 2-3 days and disappeared within 5 days. The 10 mg/kg b.w. dose was lethal causing pulmonary emphysema, heart muscle necrosis and focal hepatic necrosis. No conclusions were reported for the 15 mg/kg b.w. (data provided by industry).

Toxicity of salinomycin in ruminants has been reported in calves fed a milk powder highly contaminated with salinomycin. A recent intoxication case of 16-week old veal calves at maximum doses of 1.5 mg/kg b.w. (three times at 12 hours intervals) highlights the sensitivity of young calves, since death occurred as early as 38 hours after the first dose. Associated symptoms were vascular degeneration of the heart microfibrils and widespread tubulonephrosis (Huyben *et al.,* 2001). Another report deals with chronic exposure to salinomycin in feedlot cattle (11 weeks) at levels of 90 mg/kg in the concentrate resulting in myocardial lesions i.e. extensive myocardial fibre atrophy with multifocal hypertrophy and interstitial and replacement fibrosis (Bastianello *et al.,* 1996), the corresponding level in complete feed was not given.

4.3.4. Pigs

A 14 week subchronic study performed in 64 barrows and 64 gilts fed at 27.5, 82.5 and 137.5 mg/kg feed. The overall performance of the pigs was good and no indication of toxicity was found in this study. The authors determined a NOAEL equal to or greater than 137.5 mg/kg of feed corresponding to 5 mg/kg b.w. per day. In pregnant sows fed salinomycin at 60 mg/kg feed, no significant effects on reproductive parameters were reported (data provided by industry).

Salinomycin toxicosis was induced experimentally in weanling pigs resulting in severe ataxia and recumbency attributable to acute skeletal muscle necrosis at levels of 441 mg/kg in the coarse feed (Wendt *et al.,* 1994). This dose was approximately 10 times higher than the previous applied dose in pigs (in the EU salinomycin had been approved for the use in feed for pigs before 2006).

4.3.5. Dogs and cats

No accidental cases of salinomycin intoxication in dogs were available. However, dogs have been used in various model experiments as well as in the toxicological studies submitted in

support of the application for authorisation. Toxicological studies in dogs and other laboratory animals, used to derive the NOAEL for salinomycin sodium, are outlined in section 1.2.

An outbreak of acute paralysis in cats occurred in The Netherlands and in Switzerland related to two brands of dry cat feed from one manufacturer and was caused by contamination of the feed with salinomycin at levels of 16-21 mg/kg (van der Linde-Sipman *et al.*, 1999). The affected cats showed an acute onset of lameness and paralysis of hind limbs followed by the forelimbs. The paralysis was morphologically associated with a polyneuropathy of the peripheral nerves and characterized by primary axonal degeneration and secondary degeneration of the myelin sheath. Chemical analysis of the suspected foods, and stomach contents, liver and kidneys of affected cats confirmed the presence of salinomycin.

4.3.6. Fish

No data are available on oral toxicity of fish.

4.4. Common drug-drug interactions

Drug-drug interaction with salinomycin (used as a feed additive) and tiamulin (used therapeutically against infections with *Mycoplasma* spp.) have been frequently reported and have resulted in up to 60% mortality in some poultry herds (Lin, 1995). It has been shown that tiamulin (and also valnemulin, a related pleuromutilin) is a potent inhibitor of the activity of hepatic cytochrome P450 enzymes and particularly members of the CYP3A thus delaying the metabolism of ionophoric polyethers. Subsequently, the latter accumulate following daily ingestions, and signs of toxicity occur due to a relative overdose (Witkamp *et al.*, 1995, Szucs *et al.,* 2004).

In addition, in model experiments with male chickens for fattening (28 day-old), reared on a diet containing 60 mg/kg salinomycin with and without experimental treatment intra-oesophageally with tiamulin (50 mg/kg b.w.), the liver malondialdehyde concentration rose in the salinomycin-treated group, indicating lipid peroxidation. At the same time glutathione concentrations and glutathione peroxidase activity decreased rapidly and these effects preceded clinical signs of toxicity in the animals and indicate that salinomycin and tiamulin exert synergistic effect in affecting the antioxidant (glutathione) system (Mézes *et al.,* 1992).

The toxicity of the combination of salinomycin and tiamulin was also investigated in a two week feeding study in pigs with doses ranging from 3-30 mg salinomycin mg/kg b.w. and therapeutic application of 1-5 mg tiamulin/kg b.w. The daily dose was given by restricted feeding (twice a day) either as a bolus or mixed in the whole ration or by feeding *ad libitum*. The main clinical signs of intoxication were loss of appetite and locomotor disturbances at 8, 6 and 4 mg/kg b.w. salinomycin, and tiamulin (1-5 mg/kg). Creatine phosphokinase and

aspartate aminotransferase activities were increased in a dose–related manner in the serum. 60 mg/kg salinomycin and 20 mg/kg tiamulin were added to the feed did not induce any clinical signs of toxicity (Wendt *et al.,* 1997). A case of piglet mortality caused by the combination of salinomycin and tiamulin has also been reported in the literature (Bouwkamp and De Vries, 1991).

5. Kinetics and tissue distribution

5.1. Kinetics of salinomycin in target animal species

5.1.1. Chickens

In a study with oral and intravenous administration of a single dose of salinomycin (20 mg/kg b.w.), the absorption half-life was 0.2 hours and the elimination half-life 2 hours. The systemic bioavailability percentage was 73%. The kinetics of salinomycin can be described by a two-compartment open model with a volume of distribution at steady state (V_{dss}) of 3.3 L/kg and total body clearance ($CL_{ß}$) of 27.4 mL/kg per min. After the oral dose, a peak serum concentration of 2.48 mg/L was reached in 30 minutes and salinomycin was not detected after one day. Peak concentrations of salinomycin were reached in all the tissues studied two hours after oral dosing with concentrations of 2300, 2100, 1900, 1650, 1300 and 90 mg/kg tissue in liver, kidney, muscle, fat, heart and skin, respectively. No salinomycin residues were detected (LOQ 100 µg/kg) in tissues after 48 hours except in liver (100 µg/kg) and these had disappeared completely by 72 hours. The tissue distribution of salinomycin after feeding a dose of 60 mg/kg feed for two-weeks showed some differences. Concentrations reached in the serum and tissues of the birds were lower than those following administration of the single oral dose of 20 mg salinomycin/kg b.w. The highest concentrations were attained also in 2 hours with values of 1100, 900, 700, 670, 670 and 380 µg/kg tissue in liver, fat, heart, muscle, kidney, and skin, respectively. No residues were detected in any of the tissues after 48 hours (Atef *et al.,* 1993).

In another study, 40 male chickens for fattening received 60 mg non-labelled salinomycin/kg feed for 14 days (Kennedy *et al.,* 1995). Depletion from plasma was very rapid, falling below the LOD (0.16 µg/kg) of the ELISA method within 48 hours. Muscle and liver tissue contained salinomycin at concentrations of approximately 2.5 and 14 µg/kg, respectively, at the last day of treatment. Residue depletion was completed in muscle tissue after a 2 day withdrawal period, and in liver after 4 days (limit of detection 0.3 µg/kg tissue).

Kinetic studies assessing total radioactive residues and the parent compound, salinomycin, in chicken tissues, conducted with the maximum authorised level for complete feed (70 mg/kg, twice daily and for 7 days), indicated that the highest total radioactive residues expressed as salinomycin equivalents during the first 3-day withdrawal period are found in the liver, followed by the kidney, skin/fat and muscle. Parent salinomycin represents only a very small

and rapidly disappearing fraction of tissue residues, decreasing within 12 hours post administration to values of 1, 2 and 5 μg/kg in liver, muscle and skin plus fat, respectively (EFSA, 2004a).

Salinomycin is extensively metabolised by the chicken and unchanged salinomycin represents only a very small fraction of total radioactive residues in the excreta. More than twenty metabolites have been separated and identified from the excreta that each represents less than 10% of the total salinomycin-derived compounds. An oxidative metabolic pathway leads to mono-, di- and tri-hydroxysalinomycins and keto derivatives. Similar metabolites have been separated and identified in tissues. A considerable fraction of tissue residues is non-extractable, particularly residues in the muscle and fat. Decarboxylation of ^{14}C-salinomycin occurs to a limited but significant extent and leads to the labelling of fatty acids (and possibly proteins) (EFSA, 2004a, 2004c, 2006).

The ionophoric activity of the whole salinomycin metabolites extracted from the liver of chickens is approximately 20% that of salinomycin (EFSA, 2004a).

5.1.2. Rabbits

After oral administration to rabbits, ^{14}C-labelled salinomycin was rapidly and extensively absorbed. Elimination occurred mainly via faeces (56-80% within 3 to 8 days), and only 8-15% were recovered in urine. No label was present in the expired air. Salinomycin metabolites rapidly appeared in the bile, and hence an enterohepatic recirculation can be assumed. Maximum tissue concentration of labelled salinomycin was achieved by 24 hours in a 15-day oral dosing study. Salinomycin is metabolised in the liver yielding a broad spectrum of metabolites, mainly mono-, di- and tri-hydroxylated derivatives. Parent salinomycin was never detected in bile. The liver showed the highest amount of residues with a maximum value of 4000 μg salinomycin equivalents/kg. Radioactive labelled salinomycin was also detectable in kidney, fat, muscle and the gut wall. At the maximum authorised dose, tissue levels fell to or below the limits of detection (10 μg/kg) within 48 hours post administration. Of the four major hepatic metabolites of salinomycin, three were undetectable after 24 hours of withdrawal. The remaining metabolite, a monohydroxy-salinomycin, was still present at a concentration of 290 μg/kg salinomycin equivalents after a 12 day withdrawal period (detection limit 10 μg/kg). In the residue study at the maximum authorised level of 20 mg/kg feed, salinomycin equivalents could be detected only in the liver, and declined from about 4000 μg/kg (day 0), to 2200 μg/kg (day 2), 1300 μg/kg (day 8) and 290 μg/kg (day 12). The very slow depletion of some of the metabolites is rather uncommon for a hydrophilic compound and doubts have been raised on the true nature or origin of this residue (EC, 1992).

5.2. Kinetics of salinomycin in non-target animal species

5.2.1. Laying hens

Kinetic studies in laying hens are not available. However, Kan and Petz (2000) reviewed several studies in which residues of salinomycin were measured in eggs from laying hens that had been given salinomycin sodium in their feed. In one study, hens were fed 30, 60, 90 or 150 mg/kg of salinomycin sodium for 14 days, and the eggs contained, respectively, <10, 80, 110 and 200 μg/kg in the egg white, and 1400, 2000, 2800 and 3700 μg/kg in the yolk. Another study showed that in hens given 60 mg/kg in their feed for 7 days, excretion of salinomycin with eggs resulted in concentrations of 50 μg/kg in the egg whites, and 1500 μg/kg in the yolks. In a separate study, hens given 60 mg/kg for 5 days had salinomycin concentrations of <10 μg/kg in the egg white, and 220 mg/kg in the yolk.

Kennedy *et al.,* (1998) demonstrated in a feeding trial that the accumulation of salinomycin in eggs was 3.3 μg/kg egg per mg/kg feed. The study was performed by feeding five groups of laying hens with feed containing salinomycin at concentrations of 0.9, 1.8, 4.6, 9.1 and 13.9 mg/kg. Salinomycin was detectable in eggs from all of the groups of birds within one day at a LOD of 1 μg/kg. Salinomycin concentrations reached a plateau after approximately 9 days. Mean egg salinomycin concentrations did not exceed 60 μg/kg at any time. The results were used to test the correlation between salinomycin concentrations in the feed and in the eggs. Linear regression analysis gave the following relationship: concentration in eggs (μg/kg) = 3.33 x concentration in feed (mg/kg). Using this equation for laying hens fed a 2% (1.4 mg/kg), 5% (3.5 mg/kg) and 10% (7 mg/kg) cross-contaminated diet, concentrations of salinomycin in eggs were calculated to be 5, 12 and 23 μg/kg, respectively.

5.2.2. Pigs

Salinomycin is rapidly and effectively absorbed by pigs when administered by the oral route. An average of 83.5% of the radioactivity was excreted in the faeces with little intact salinomycin being present. About 2.1% of the given dose was excreted with urine. After 4 days, total radioactive residues in the liver were 100 μg/kg and below the LOD of 10 μg/kg in muscle, kidney and fat. Pig bile contains mainly the di- and trihydroxylated derivatives, while liver also contains other hydroxylated products, notably the monohydroxy derivative also found in rabbit liver. After administration of a single dose to pigs, 71%-88% were found in the gastro-intestinal tract after 12 hours, and radioactivity was only detectable in the liver. Repeated dose administration produced residue levels of 1.5 mg/kg in the liver 8 hours after withdrawal, decreasing to 0.4 mg/kg after 12 hours (monohydroxy-salinomycin absent, only di- and trihydroxy-salinomycin present), 0.2 mg/kg after 24 hours and 0.06 mg/kg after 60 hours, when analysed by a radiochemical method with a limit of detection of 0.01 mg/kg (EC, 1991). However, the dose level and duration of the study were not reported in the SCAN report and were not available to the CONTAM panel.

Similar results were obtained in the study of Dimenna *et al.* (1990). Pigs were fed with salinomycin at 41 mg/kg in the diet for 29 days and then dosed with ^{14}C-labelled salinomycin at 41 mg/kg in the diet by a feeding regimen at 12 hour intervals for 8 days. Eight hours after the final dose of ^{14}C-salinomycin, total radioactive residues were below the LOQ of 5 µg/kg in kidney, fat and muscle but 1800 µg salinomycin equivalents/kg in liver. By linear extrapolation, a concentration of 60, 150 and 300 µg salinomycin equivalents/kg in liver would be anticipated if the pigs had been given feed cross-contaminated at a level of 2, 5 and 10%, respectively. Parent compound, salinomycin, in the liver samples accounted for <1% of the total radioactive residue in all samples. In swine, salinomycin is extensively metabolised, resulting in numerous metabolites in the liver, which have not been identified or were not present in sufficient quantity to be useful as a marker compound. Approximately 15-20% of the total residue in liver was bound. Ionophoric activity in extracts of livers from the treated pigs was minimal with only 10% of the ionophoric activity of an equivalent amount of salinomycin (Dimenna *et al.,* 1990).

5.2.3. Cattle

Total radioactivity levels of salinomycin in edible tissues of cattle following oral administration of ^{14}C-labelled salinomycin sodium of 0.9 mg/kg b.w per day were below the LOQ of 59 µg/kg in kidney, muscle and fat. Only in the liver, an average concentration of salinomycin equivalents of 2263 and 1548 µg/kg was measured 12 and 36 hours after the last dose (data provided by industry).

5.2.4. Other non-target animal species

No kinetic studies were available for the non-target animal species other than laying hens, pigs and cattle.

No information was available on the potential for salinomycin to leave residues in milk.

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 cases of intoxication and the limited number of toxicological investigations indicate significant species differences in the susceptibility to salinomycin and a small margin of safety. In chickens, the major target animal species, considerable growth rate depression was seen at concentrations in feed exceeding the maximum authorised level

for chickens for fattening by a factor of approximately two (150 mg/kg feed) (see 4.2.1.). In rabbits, the second target species, embryotoxicity (increased number of resorption sites) occurred at a dietary level of 16 mg/kg, (equivalent to 0.63 mg/kg b.w. per day). This is below the maximum authorised level for this animal species (25 mg/kg). In addition, one field-trial found reduced litter size at a dietary level of 25 mg/kg (although this effect was not seen in other field-trials that used levels of 20-75 mg/kg). The current authorisation of salinomycin used as coccidiostat in rabbits permits use only in rabbits for fattening. The SCAN noted that if a future applicant were to request authorisation of use in rabbits for breeding, it would need to demonstrate an absence of reproductive toxicity in rabbits at proposed dose levels (EC, 1992).

Turkeys are particularly sensitive and accidental exposure of turkeys to feed intended for chickens for fattening can cause mortality (see 4.3.1). The same applies to horses, and cases of fatal intoxications have been described following ingestion of feed containing salinomycin at the level authorised for chickens for fattening (corresponding to an exposure between 0.12 and 0.25 mg/kg b.w).

Following repeated exposure of cattle to 84 mg/kg salinomycin in feed (1.2× the maximum authorised level for chickens for fattening) extensive myocardial lesions were observed. In pre-ruminant calves fatal intoxications are described. In contrast, pigs seem to have a high tolerance for salinomycin. Experimental data and case reports describe also the sensitivity of cats and dogs to salinomycin below the maximum authorised levels for chickens for fattening (70 mg/kg feed).

In conclusion, accidental ingestion of feed intended for chickens for fattening containing the maximal authorised level of 70 mg salinomycin/kg feed comprises 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 limited data available on the occurrence of salinomycin in feed materials for non-target animal species show values between 0.07 and 6.1 mg/kg feed, corresponding to a rate of cross-contamination between 0.01 and 8.7% of the maximum authorised level for chickens for fattening (70 mg/kg feed). However, incidental very high values, even exceeding the maximum authorised level have also been found, indicating errors in the allocations of feed batches or mixing errors.

In accordance with the mandate, levels of cross-contamination of 2%, 5% and 10% of the maximum level authorised for target animal species (corresponding to 1.4, 3.5 and 7 mg salinomycin per kg feed, respectively) have been evaluated. At an average feed consumption

of 50 g/kg b.w. in monogastric species, these concentrations would result in doses of approximately 0.1, 0.2 and 0.35 mg/kg b.w., respectively. These are below the overall NOAEL of 0.5 mg/kg b.w. identified in experimental animals. However, horses are more sensitive (with lethal doses reported at doses as low as 0.12-0.25 mg/kg b.w). The Panel concluded that toxic effects in horses could result from cross-contamination of feed at levels above 2%. For all other non-target species, the Panel concluded that adverse health effects are unlikely to result from cross-contamination of feed up to a level of 10% of the maximum authorised level for chickens for fattening.

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

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

Results from analyses of eggs in the Member States show occasionally occurrence of low levels and only seldomly high levels are found, the highest concentration being 141 µg/kg in eggs, which, according to the calculations of Kennedy *et al.* (1998), could result from laying hens fed 43 mg salinomycin/kg feed. The results of the analyses in the Member States show that residues are occasionally found in liver of target animals. From the UK survey the highest concentration found in a non-target animal was 4 µg/kg in sheep liver.

The deposition of salinomycin equivalents in eggs (particularly in yolks) was demonstrated by Kan and Petz (2000) indicating negligible amounts of salinomycin in the egg white (albumen). Using the Kennedy *et al.* (1998) study and the correlation between salinomycin levels in feed and eggs, the Panel estimated that concentrations from cross-contaminated feed at a level of 2%, 5% and 10% (1.4, 3.5 and 7 mg/kg) would result in concentrations of salinomycin in eggs of approximately 5, 12 and 23 µg/kg, respectively.

Based on a residue distribution study in pigs it was estimated by linear extrapolation that cross-contaminated feed at a level of 2%, 5% and 10% (1.4, 3.5 and 7 mg/kg) would result in liver residues of approximately 60, 150 and 300 µg salinomycin equivalents/kg tissue, respectively. Studies have shown that the parent salinomycin only represents 1% of the salinomycin equivalents in liver. It was also reported that 15-20% of the liver residues were bound (i.e. unextractable) and that the ionophoric activity of the extractable residue was 10% of the ionophoric activity of salinomycin. Thus, the ionophoric activity of the residues of 1800 µg salinomycin equivalents/kg liver were equal to the ionophoric activity of 153 µg/kg of salinomycin (i.e. 10% of 80-85% of 1800 µg/kg) and the estimated ionophoric activity of

the residues resulting from 2, 5 and 10% contamination of feed would be equal to that of 5.2, 13 and 26 μg/kg of salinomycin, respectively.

No other animal tissues have been reported to contain residues of salinomycin, despite the slow depletion of residues in fatty tissues as described for poultry. In addition, no data are available on the possible carry-over into dairy 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 salinomycin from cross-contaminated feed are 100 g of eggs and 100 g of liver[20].

As mentioned above a 2, 5 and 10% cross-contamination of feed was estimated to result in concentrations of approximately 5, 12 and 23 μg/kg, respectively. Consumption of 100 g of eggs would result in an intake of about 0.5, 1.2 and 2.3 μg, which is equivalent to 0.008, 0.02 and 0.038 μg/kg b.w. for a person of 60 kg b.w. This corresponds to about 0.2, 0.4 and 0.8% of the ADI of 5 μg/kg b.w. as derived by the FEEDAP panel.

By using the kinetic data to calculate residues in pig liver, a 60 kg consumer eating 100 g liver per day would ingest 6, 15, 30 μg of salinomycin equivalents, corresponding to 0.1, 0.25, 0.5 μg salinomycin equivalents/kg b.w. which is 2, 5 and 10% of the ADI respectively. By using the data on ionophoric activity of salinomycin equivalents the exposure would be 0.0086, 0.021, 0.043 μg/kg b.w. which is 0.17, 0.42, 0.85% of the ADI.

The highest concentration of salinomycin found in non-target animal species was 4 μg/kg in sheep liver, which would contribute to the exposure with 0.007 μg/kg b.w. corresponding to 0.13% of the ADI.

The above estimates are all well below the ADI of 5 μg/kg b.w as derived by the FEEDAP Panel. In addition, it was recognised that consumer exposure to residues of salinomycin resulting from cross-contamination of feed is likely to be infrequent.

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

[20] Values for daily human food consumption, as defined in Directive No (EC) 2001/79[20] 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

CONCLUSIONS

- Salinomycin exerts signs of toxicity typical of ionophoric compounds in various non-target animal species, including turkeys, horses, pre-ruminant calves, cats and dogs. Intoxications can be fatal, and may occur in sensitive animal species at feed concentrations well below the maximum levels authorised for use in chickens for fattening (70 mg/kg feed).
- Toxic effects in horses could result from cross-contamination of feed at levels exceeding 2%. For all other non-target species, adverse health effects are unlikely to result from cross-contamination of feed up to a hypothetical level of 10% of the maximum authorised level.
- The available kinetic data indicate that salinomycin residues would primarily occur in eggs and liver tissue.
- Calculations based on data from feeding trials and kinetic experiments indicate that the maximum level of human exposure from salinomycin residues would be 0.5 µg salinomycin equivalents (total radioactive residues)/kg b.w. from pig liver and 0.038 µg/kg b.w. from eggs from feed cross-contaminated at a hypothetical level of 10% of the maximum authorised level.
- The estimated dietary exposure of consumers was well below the ADI of 5 µg/kg b.w. as established by the FEEDAP Panel. Therefore, the Panel concludes that there is negligible risk to consumers' health from the ingestion of salinomycin 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 salinomycin 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 salinomycin should be encouraged as no data are available at present.

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