

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

Scientific Opinion of the Panel on Contaminants in the Food Chain

(Question N° EFSA-Q-2005-220A)

Adopted on 20 September 2007

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SUMMARY

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

Narasin 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 with a maximum content of the active substance in feed of 70 mg/kg and a withdrawal period of one day. 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

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 narasin authorised for use as a feed additive, *The EFSA Journal* (2007)552, 1-35

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.

Signs of intoxication, including anorexia, dyspnoea, lung oedema, liver cell necrosis and muscle fibre damage have been reported to occur in various non-target animal species. These signs of toxicity are consistent with the mode of action of polyether ionophores. Particularly sensitive are dogs, horses, cattle and probably turkeys and rabbits. Toxicity occurred in turkeys and rabbits at feed concentrations lower than the maximum level authorised for chickens for fattening. This reflects the considerable species differences in sensitivity and the small margin of safety between the effective dose of narasin as a coccidiostat and the dose that causes toxicity. The Panel concluded that in sensitive non-target animal species adverse effects may occur at feed concentrations below the maximum level authorised for use in chickens for fattening.

In contrast, it is expected that no toxicological or pharmacological effects will occur in non-target animals given feed containing narasin at the dietary levels resulting from cross-contamination up to 10 % of the maximum amount permitted in the feed of target animals. This expectation is based on a comparison of the level of exposure of non-target animal species to narasin via contaminated feed batches with the no-observed-adverse-effect-level (NOAEL) derived from toxicological and pharmacological studies. In dogs, the most sensitive laboratory animal species, a NOAEL of 0.5 mg/kg b.w. was identified, based on the induction of neurotoxicity in a one-year feeding trial. Assuming a feed intake of approximately 50 g/kg b.w. per day, which is applicable to most monogastric food-producing animals and levels of cross contamination of 2, 5 and 10 %, this would result in levels of exposure of 0.07, 0.17 and 0.35 mg/kg b.w. per day, respectively, which are all below the NOAEL.

Kinetic studies in various animal species showed that narasin does not accumulate in edible animal tissues. When given to laying hens it can be found in eggs. The highest levels of narasin residues found in edible tissues of non-target animals in kinetic studies (pig liver and chicken eggs) were used to calculate a worst-case estimate of consumer exposure to narasin. No data on possible carry-over into milk are available.

These calculations indicated that human exposure estimated to result from consumption of food products from non-target animal species exposed to feed cross-contaminated up to a level of 10 %, would be well below the acceptable daily intake (ADI) of 5 µg/kg b.w. as established by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel). Therefore, the Panel concluded that there is negligible risk to consumers' health from ingestion of narasin residues in tissues of animals exposed to feed cross-contaminated up to a level of 10 %.

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

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BACKGROUND AS PROVIDED BY REQUESTOR

1. Cross-contamination

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

Cross-contamination in purchased premixtures

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

Product related cross-contamination

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

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

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

Establishment related cross-contamination

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

2. Legal provisions as regards minimisation of cross-contamination

Directive No (EC) 95/69²

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

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

intermediaries putting these products into circulation must be approved. The approval is subject to compliance with the minimum conditions laid down in the Annex.

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

Regulation No (EC) 183/2005³

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

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

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

(a) permit adequate cleaning and/or disinfection;

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

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

Article 3 of Council Directive No (EC) 70/524⁴ concerning additives in feedingstuffs provides that no additive may be put into circulation unless a Community authorisation has been granted. This Community authorisation can only be granted if, taking into account the conditions of use, it does not adversely affect human or animal health or the environment, nor harm the consumer by impairing the characteristics of animal products.

³ OJ L 35, 8.2.2005, p. 1

⁴ OJ L 270, 14.12.1970, p.1

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

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

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

| Coccidiostat (active substance) | Species or category of animals for which the use of coccidiostats is authorised (target animal) | Authorised maximum content of narasin in complete feed |
|--|--|---|
| Narasin | Chickens for fattening | 70 mg/kg (Monteban TM) ⁶ |

4. Unavoidable cross-contamination (under practical conditions)

Narasin 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 narasin can result in cross-contamination to feedingstuffs for non-target animal species.

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

5. 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 (EC) 2002/32⁷ of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

⁵ OJ L 268, 18.10.2003, p. 29–43

⁶ Another feed additive product that contains narasin in combination with another coccidiostat is also on the market. The continued use of this product has yet to be evaluated by FEEDAP. Its use is not taken into consideration in this Opinion on narasin in feed of non-target animals.

⁷ 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)

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

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

TERMS OF REFERENCE AS PROVIDED BY REQUESTOR

In accordance with Article 29 (1) (a) of Regulation No (EC) 178/2002⁸ the Commission asks EFSA to provide an opinion on the risks involved for animal health and public health as the consequence of undesirable cross-contamination of narasin authorised as feed additive into non-target feeds.

The assessment should take into account hypothetical cross-contamination rates of 2 %, 5 % and 10 % from feed produced with the highest authorised dose of narasin 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 narasin into feed for non-target animals,
- on the basis of the available information, an estimate of the level of residues present in food of animal origin from non-target 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.

⁸ OJ L 31, 1.2.2002, p. 1–24

ACKNOWLEDGEMENTS

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

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

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

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

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

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

Target animal species: Animal species or animal category (age group) within a species for which the compound under consideration is licensed for use as a coccidiostat.

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

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

Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of the data provided by the applicant.

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

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

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

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

ADI values: Acceptable daily intake 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 CVMP⁹ or the JECFA¹⁰) is used for the risk characterization. The CONTAM Panel noted in some cases the divergence between ADI values derived by the FEEDAP Panel and the ADI values derived by the CVMP and/or JECFA. These differences were attributable to the application of different uncertainty 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) 1055/2006¹¹ amending the Annexes I and III of Regulation No (EC) 2377/90¹² to propose maximum residue limits (MRLs) for a number of coccidiostats. However, none of the substances under consideration

⁹ The Committee for Medicinal Products for Veterinary Use of the European Medicines Agency

¹⁰ 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).

¹¹ OJ L 192, 13.7.2006, p. 3–5

¹² OJ L 224, 18.8.1990, p. 1–8

are licensed at present as veterinary medicinal product. The FEEDAP has also recommended MRLs for some coccidiostats.

Residues of coccidiostats in edible tissues, milk and eggs: According to Directive No (EC) 96/23¹³ Member States are obliged to monitor certain substances and residues thereof in animals and animal products. These data are collected by the Commission and a compilation of the results from 2004 and 2005 are used in the human exposure assessment.

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

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

ASSESSMENT

1. Introduction

Narasin belongs to the polyether monocarboxylic acid class of ionophores produced by *Streptomyces aureofaciens* strain NRRL 8092. It exerts both anticoccidial and antimicrobial effects. According to EFSA (2004) at least 85 % of the narasin activity should be due to narasin A. HPLC analysis of the composition of one lot of Monteban G100, as an example, indicated that the narasin material in the later consisted of 96 % narasin A, 1 % narasin B, 2 % narasin D and 1 % narasin I. The relative bio-potency of the structural variants of narasin is 1, 0.25, 1.4 and 0.01, respectively (EFSA, 2004). The chemical structure of narasin A is presented in Figure 1.

¹³ OJ L 125, 23.5.1996, p. 10–32

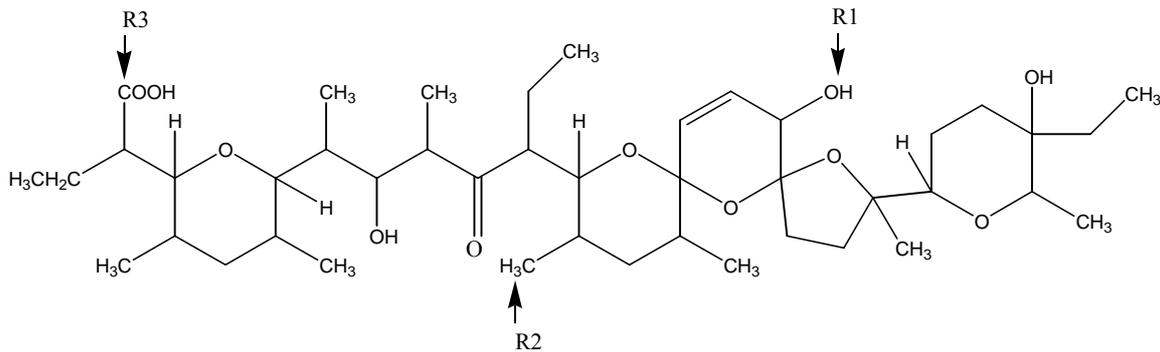


Figure 1. Chemical structure of narasin A. Structural variants: narasin B: R1 = =O, R2 = -CH₃, R3 = -COOH, narasin D: R1 = -OH, R2 = -C₂H₅, R3 = -COOH, narasin I: R1 = -OH, R2 = -CH₃, R3 = -COOCH₃.

The IUPAC name of narasin A is α -ethyl-6-[5-[2-(5-ethyltetrahydro-5-hydroxy-6-methyl-2H-pyran-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]tetrahydro-3,5-dimethyl-2H-pyran-2-acetic acid and the CAS number is 55134-13-9. The molecular weight is 764 and the molecular formula is C₄₃H₇₂O₁₁ (EFSA, 2004).

The water solubility of narasin is 102 mg/L at pH = 7 and 681 mg/L at pH = 9. The pKa value is 7.9, Log Kow >6.2 and Log Koc 6.06 - 6.88 (EFSA, 2004).

As summarised already in the Terms of Reference, narasin is authorised as a coccidiostat to be used in feeds for chickens for fattening at a minimum-maximum concentration of 60 - 70 mg/kg feed and a withdrawal period of one day before slaughter (Regulation No (EC) 1464/2004¹⁴). In addition, a combination product containing narasin and nicarbazin is authorised for use in feed for chickens for fattening with a minimum-maximum narasin concentration of 40 - 50 mg/kg (Regulation No (EC) 70/524¹⁵). In the current opinion this combination product will not be considered, as the concentration of narasin is lower than the concentration authorised for narasin as a single additive.

1.1. Biological activity of narasin

Anticoccidial activity

Narasin, like other polyether ionophores, is effective against sporozoites, and early and late asexual stages of coccidia in the intestines of chickens. The biological activity of ionophoric coccidiostats such as narasin is based on their ability to form lipid soluble and dynamically reversible complexes with ions. Narasin preferentially forms complexes with monovalent cations such as the alkaline ions K⁺ and Na⁺. Polyether ionophores function as carriers of ions, mediating an electrically neutral exchange-diffusion type of ion transport across the membranes. The resultant changes in the

¹⁴ OJ L 94, 1.4.2006, p. 26–27

¹⁵ OJ L 270, 14.12.1970, p. 1 / C 50, 25.2.2004, p. 1

transmembrane ion gradient and the electrical potential produce critical effects on cellular functions and the metabolism of coccidia (and also of mammalian cells) (EFSA, 2004).

Antibacterial activity

The antibacterial spectrum of activity of narasin is limited to Gram-positive bacteria, including *Enterococci*, *Staphylococci* and *Clostridium perfringens*. The minimum inhibitory concentrations (MICs) for *Staphylococcus aureus* and *Enterococcus faecalis* range between 0.39 to 100 µg/mL and from 0.2 to 100 µg/mL, respectively. MICs for *Clostridium* ranged from 0.1 to 4.0 µg/mL. Gram-negative bacteria are, in general, resistant to narasin with MICs > 512 µg/mL (EFSA, 2004).

Enterococci may develop resistance to narasin. In the monitoring programmes, resistance to the ionophores, salinomycin and narasin for broiler isolates of *E. faecalis* and *E. faecium* is reported. There is no cross-resistance to other commonly used antimicrobials except to salinomycin. It is unlikely that narasin increases *Salmonella*-shedding at the authorised inclusion level (EFSA, 2004).

Narasin and other ionophore compounds have never been used in human medicine and hence the drug is not classified as a critically important antibiotic for human use by the WHO expert panel (2005). It has, however, been classified by the Office Internationale des Epizooties (OIE, World Organization for Animal Health) (OIE, 2006) as a very important antibiotic for veterinary medicine for control of coccidiosis. Narasin is also effective against necrotic enteritis in poultry.

The effect of narasin 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 narasin

The general underlying pharmacological and toxicological properties of narasin are related to its structure as an ionophoric compound, affecting ion transport across cell membranes, where it forms reversible cation complexes.

A full toxicological assessment of narasin has been performed by the FEEDAP Panel (EFSA, 2004). A brief summary of the data presented in the FEEDAP opinion is presented here, as the data submitted to the FEEDAP panel were not available to the CONTAM panel. The acute oral toxicity of narasin is low: oral LD₅₀ values of 15.8 - 16.7 mg/kg b.w. in mice and 18.5 - 21.1 mg/kg b.w. in rats have been reported. Data from various animal species indicate that acutely toxic doses of narasin induce anorexia, hypoactivity, leg weakness, ataxia, and diarrhoea. These clinical symptoms appear often after a lag period of one or more days, depending on the dose, and are reversible. Other signs of toxicity include myocardial necrosis, resulting in an impairment of cardiac function with secondary lung oedema and dyspnoea. Narasin was neither mutagenic nor carcinogenic, and did not produce reproductive or developmental toxicity. Maternal toxicity was shown in rabbits at levels above 1 mg/kg b.w. per day. The critical toxicological study used by the FEEDAP Panel to set the ADI was a one-year study in dogs treated orally with narasin in gelatine capsules at the doses of 0,

0.5, 1.0, 2.0 mg/kg b.w. per day. At termination, the animals were given comprehensive pathological examinations and treatment-related changes were found at 1 and 2 mg/kg b.w per day in both males and females with focal degeneration of skeletal muscles, including the diaphragm, and axonal degeneration of peripheral nerves. A NOAEL value of 0.5 mg/kg b.w was identified from this dog study, and an ADI of 0.005 mg/kg b.w. was calculated by applying a 100-fold uncertainty factor (Novilla *et al.*, 1994, EFSA, 2004).

In contrast to some other ionophoric polyethers, regulatory authorities have not established a microbiological ADI value for narasin, although *in vitro* MIC (minimum inhibitory concentration) data showed that narasin possessed antibacterial properties¹⁶.

For setting the MRL, the FEEDAP Panel considered the rapid metabolic conversion of narasin into a large number of individual metabolites of narasin and the low concentrations of residues in tissues. The toxicological risk due to the metabolites was assumed to represent a risk to consumers that was less than or equal to that of narasin itself. Consumer exposures was then determined using values for daily human food consumption, as defined in Directive No (EC) 2001/79¹⁷ (for chickens: 300 g muscle, 100 g liver, 10 g kidney, 90 g skin/fat in natural proportions and 100 g eggs). The highest residue levels resulted in an estimated dietary exposure of 59 µg narasin equivalent (total radioactive residues) per day corresponding to 20 % of the ADI. These residues correspond mostly to narasin A since this compound represents quantitatively 96 % of all the isomers. Based on the FEEDAP assessment, a uniform MRL of 50 µg/kg wet tissue was proposed for residues of narasin in liver, kidney, muscle and skin/fat with narasin A as the marker substance (EFSA, 2004), and established by Regulation No (EC) 545/2006¹⁸.

1.3. Cross-contamination of feed

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

¹⁶Regulatory authorities that evaluate veterinary medicines are required to evaluate the possible effects of microbiologically active substances on the bacterial populations of the human gut flora and to identify a microbiological ADI.

¹⁷ OJ L 267, 6.10.2001, p. 1–26

¹⁸ OJ L 94, 1.4.2006, p. 26-27

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 batch 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 among others for narasin (Kennedy *et al.*, 1996, 1998; Mc Evoy *et al.*, 2003; Noser *et al.*, 2006). The authors concluded that:

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

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

1.4. Specific data on narasin-based feed additive products

The stability of narasin at 80 g/kg in premixes was studied in three experiments, where samples were stored at different temperatures (4, 25, 40 and 60 °C) and the remaining narasin measured at different intervals. Losses did not exceed 10 %, when samples were stored for three years at 25 °C

or up to 18 months at 40 °C. The stability and homogeneity (narasin at 70 mg/kg) were studied in expanded and pelleted feeds. Good homogeneity and no loss of activity as a result of expanding and pelleting were confirmed during three months storage at 25 °C for common feeds (EFSA, 2004).

The dusting potential of the narasin-containing product Monteban G100 was investigated using a Lilly Dust Dispenser. It was claimed that the results obtained, using these methods, are qualitatively similar to those obtained using the common Stauber-Heubach method. Results indicated that Monteban G100 is a free flowing granular material (85 % > 100 µm) with small portions of particles < 10 µm (0.05 %), 0.36 % by mass and about 0.18 % of narasin activity became airborne (EFSA, 2004).

In another study, eleven samples of Monteban 10 % premix were evaluated by the Stauber-Heubach method. On average, 0.05 % of the product formed dust particles of a particle diameter 7.2 µm or less, and 0.2 % formed particles of 19 µm or less (EFSA, 2004). Taken together, these results indicate that the dusting potential of the commercial product Monteban is limited.

2. Methods of analysis for narasin

2.1. Analysis of narasin in premixtures and animal feeds

In the process of approval of a feed additive containing narasin, the applicant presented an analytical method for quantification of narasin A in supplemented feed. However, several other methods are in use in Member States.

The method presented by the applicant (EFSA, 2004) is a high performance liquid chromatography (HPLC) method, with post-column derivatisation with vanillin, which has been used and validated for the quantification of narasin in chicken premixes and feeds. The chromatographic conditions were selected in order to separate narasin from matrix components and from other polyether ionophores.

The limit of detection (LOD) and limit of quantification (LOQ) for narasin A were 0.05 mg/kg and 0.1 mg/kg, respectively. Intra-laboratory analysis using several lots of feed indicated a relative standard deviation (RSD) of 2.4 to 5.6 % in feed rations, the recovery being around 93 - 98 %.

A microbiological assay using *Streptococcus faecium* and a turbidimetric measurement were also made available.

In addition, Thalmann *et al.* (2004) described a reversed-phase liquid chromatography method for the analysis in feedingstuffs, which was validated in an inter-laboratory study. In this method, a methanol/buffer was used as the extraction solvent and narasin was detected at 600 nm after post-column derivatisation with dimethylamino-benzaldehyde. Recovery was > 90 %. The reproducibility in feed (20 - 140 mg/kg) ranged between 1.2 and 10.5 %; and the within-laboratory

reproducibility (RSDR) ranged between 2.2 and 4.9 %. In an inter-laboratory study, 5 broiler feeds (4 positive, 1 blank) and 1 premix were analysed in 13 different laboratories. The RSD for the feedingstuffs (20 - 120 mg/kg) varied between 2.17 and 7.57 %. LOQ and LOD were lower than 0.5 mg/kg and 0.25 mg/kg, respectively (Thalmann *et al.*, 2004).

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

In the analytical procedure described by Dusi and Gamba (1999), narasin was extracted from feed samples using methanol and (without a clean-up step) directly derivatised with 2,4-dinitrophenylhydrazine. The derivatisation mixture was analysed using ultraviolet (UV) detection at 305/392 nm. The recoveries of narasin from spiked samples were 85 – 100 % with RSDs of 4 – 10 % in a concentration range of 50 – 150 mg/kg. The LOD (S/N = 3) was 20 mg/kg

In conclusion, it can be stated that the methods of Thalmann *et al.* (2004) and Turnipseed *et al.* (2001) are sensitive enough to detect the low levels of narasin which might result from cross-contamination, i.e. 1 – 10 % of the prescribed level of 60 – 70 mg/kg complete feed, and could be used for official control purposes, as well as the method presented by the applicant.

2.2. Analysis of narasin residues in animal products

An analytical high performance liquid chromatography (HPLC) method was described and validated by the applicant for the determination of narasin in chicken tissues with an LOQ of 0.025 mg/kg and a LOD of 0.01 mg/kg (EFSA, 2004).

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), narasin residues are analysed in meat by 14 and in eggs by 12 out of 20 NRL. The Member States used different methods such as enzyme immunoassay (EIA), HPLC, high performance thin layer chromatography (HPTLC), LC-MS and LC-MS/MS for screening and confirmatory purposes. LC-MS or LC-MS/MS are the most commonly used methods. The decision limits ranged between 1 and 200 µg narasin/kg tissue.

There is no minimum required performance level (MRPL) established for narasin 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 the presence of narasin in animal products. The LODs were 0.56 and 0.28 µg/kg for muscle and eggs, respectively.

Heller *et al.* (2004) described a screening method based on Ion-Trap LC-MS/MS for non-polar residues in eggs. The LOD was 1 µg/kg for narasin in eggs.

2.2.2. Quantitative and confirmatory methods

A liquid chromatography with absorbance detection method (LC-UV-Vis) was proposed by Ward *et al.* (2005) to control narasin residues in chicken kidney, liver, muscle and skin/fat. The LODs for these tissues were 3.2, 2.6, 3.5 and 2 µg/kg, respectively.

Rosen (2001) described a multi-residue method based on LC-MS/MS for survey of narasin residue in eggs and liver with a LOD estimated to be 0.027 µg/kg.

A multi-residue method based on LC-MS was reported by Hormazabal and Yndestad (2000). The LODs for narasin were 5, 3, 3 and 2 µg/kg in eggs, fat, liver and muscle, respectively.

Matabudul *et al.* (2002) presented a multi-residue LC-MS/MS method, which was developed to quantify narasin residues in sheep and chicken liver and eggs. The LOD was 1 µg/kg for all matrices.

Dubois *et al.* (2004) described a multi-residue qualitative method based on LC-MS/MS for the determination of nine coccidiostats in muscle tissue and eggs. For narasin residues in muscle tissue, the decision limit ($CC\alpha$)¹⁹ was 0.3 µg/kg. The method is also applicable to eggs.

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

Another multi-residue method based on LC-MS/MS was described by Rokka and Peltonen (2006) for the quantitative determination of four coccidiostats (lasalocid, monensin, salinomycin and

¹⁹Definitions 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 α 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 β . $CC\alpha$ 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).

narasin) in eggs and broiler meat. The CC α for narasin were 0.8 and 1.6 $\mu\text{g}/\text{kg}$ in eggs and muscle tissue, respectively.

3. Occurrence of narasin

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

Data on cross-contamination of feed are scarce. The Czech Republic reported the results of 254 analyses that were performed during 2006. Only 1 positive sample was found. This was a premixture for pigs that contained 45.3 mg narasin/kg. The LOD of the analytical method was 0.5 mg/kg.

Denmark reported the analyses of 111 samples of feeds that were sampled between 2004 and 2007. The samples were all taken from the first batch of feed that was intended for non-target animal species that was prepared following the production of feed for target animal species and a cleaning procedure. Three positive samples, containing 0.015, 0.035 and 0.6 mg narasin/kg feed were found.

Information from the Rapid Alert System for Food and Feed (RASFF)²⁰ that was collected between April 2002 and April 2006 showed 12 incidents in which narasin was found in feed for non-target animal species (data provided by the European Commission). The amounts detected were between 0.12 and 3.6 mg narasin/kg feed.

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

Residues of narasin 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 animal species are analysed by the Member States according to requirements in Directive No (EC) 96/23²¹. 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 μg narasin/kg tissue as their non-compliant limit²².

For narasin, combined results of 2004 and 2005 show that no non-compliant samples were found amongst 12084 samples of different animal tissues. The samples were distributed as follows: 3724 samples of eggs, 3889 samples of poultry, 1569 samples of bovine, 1326 samples of pigs, 1028

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

²¹Directive 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.

²²After April 2006, levels above the MRLs has been defined as non-compliant for chicken muscle, liver and skin/fat

samples of sheep/goat, 344 samples of farmed game, 274 samples of rabbits and 22 samples of horses. The LOD ranged from 0.03 to 100 µg/kg.

The Swedish Food Administration has summarised the occurrence of narasin in eggs from 1999 to 2005 (Livsmedelsverket, 2006; Table 2). The Swedish results above the LOQ in 2004 and 2005 were apparently not reported to the Commission as non-compliant, and the highest value found in these two years was 6.6 µg/kg.

Table 2. Swedish results from 1999 to 2005 of narasin residues in egg (Livsmedelsverket, 2006)

| | 1999 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 |
|---|--------------|--------------|--------------|--------------|---------------|--------------|-----------------|
| Narasin not detected or not confirmed | 12 (50 %) | 70 (48 %) | 69 (49 %) | 35 (25 %) | 105 (75 %) | 68 (48 %) | 108 (76.6 %) |
| Narasin confirmed, levels below LOQ (0.5 µg/kg) | 2 (8 %) | 41 (28 %) | 39 (28 %) | 41 (30 %) | 28 (20 %) | 38 (27 %) | 32 (22.7 %) |
| Narasin confirmed, levels above LOQ (0.5 µg/kg) | 10 (42 %) | 35 (24 %) | 32 (23 %) | 63 (45 %) | 7 (5 %) | 35 (25 %) | 1 (0.7 %) |
| Highest value found (µg/kg) | 10.6 | 10.8 | 10.7 | 8.5 | 3.4 | 6.6 | 1.9 |
| Total amount of samples analysed | 24 | 146 | 140 | 139 | 140 | 141 | 141 |

In Denmark, 182 egg samples were analysed from 2004 to 2006. One sample contained 1.8 µg narasin/kg with a LOD of 0.1 µg/kg. In Belgium, no narasin was found with a LOD of 2 µg/kg amongst 958 samples of muscle tissue from different animal species and eggs in 2005 and 2006.

Narasin has been analysed in United Kingdom surveys for residues of veterinary drugs in foods (UK-VMD, 1995-2005). Since 2000, narasin has been included in the survey program as part of a general screen for various ionophores in certain foods. Prior to this date, only individual results for narasin in certain foods were reported. Narasin was not detected in any food samples taken between 1995 and 2005. The foods tested were chicken eggs (2133 eggs tested); livers of chickens for fattening (1837 samples tested), hens (93), chickens (not specified) (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). The LOQs for the analytical methods varied between 1 and 50 µg/kg (UK-VMD, 1995-2005). No data were available for narasin residues in milk.

In a survey of 320 egg samples, purchased in eight different European countries, eggs were analysed for the presence of nine different coccidiostats including narasin. Narasin was found in

four samples, accounting for 2.9 % of all positive samples, all four results were below 10 µg/kg. The CC α was 1 µg/kg (Mortier *et al.*, 2005b).

4. Toxicity of narasin

4.1. Mechanisms of toxicity

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

4.2. Toxicity of narasin in target animal species

4.2.1. Chickens

The toxicity of narasin for chickens for fattening has been reviewed and summarised recently by the FEEDAP Panel. The tolerance studies submitted by the applicant suggested that levels of 120 mg/kg feed or higher can cause a reduction in body weight gain, increased mortality and a reduction in reproductive performance. No adverse effects were observed at a level of 100 mg/kg feed. Consequently, the FEEDAP Panel concluded that the margin of safety at the maximum level of narasin authorised for target species is 100/70 (1.4). A very small safety margin is common for ionophores (EFSA, 2004).

4.3. Toxicity of narasin in non-target animal species

4.3.1. Laying hens

As narasin is licensed for the use in chickens for fattening, it is possible that feed intended for chickens for fattening might be (accidentally or intentionally) given to laying hens. Therefore, the effects of narasin (and other anti-coccidial agents) on the production and reproduction of White Leghorns chickens were evaluated (Jones *et al.*, 1990a). Narasin given at a dose of 70 mg/kg of feed (the maximum authorised dose level) did not affect egg production, egg weight, shell thickness or internal egg quality. In another experiment (Jones *et al.*, 1990b), narasin adversely influenced egg weight to a minor degree and reduced hatchability at a level of 70 mg/kg feed but did not have any effect on egg production or fertility.

4.3.2. Turkeys

Clinical signs of intoxication and high mortality rates (over 32 %) were reported from a turkey flock of 3000 birds aged 11 weeks (Sályi *et al.*, 1988). The feed consumed by the turkeys contained 42.8 mg/kg narasin, and was at the same time slightly contaminated with monensin. The most characteristic clinical signs were locomotor disorders, which were accompanied by severe dyspnoea and moderate diarrhoea. At necropsy, muscle fibre damage appeared, in most cases visible macroscopically in most of the decedent birds. Histologically, the lesions were found to be Zenker's degeneration and necrosis. In addition, catarrhal enteritis, renal degeneration and pulmonary congestion were observed. Nearly one-third of the flock died within one week after the appearance of clinical signs.

4.3.3. Ducks

Mallard ducks were given narasin at a concentration of 0, 200, 560, 1800 or 5000 mg/kg of feed for five days followed by three days of feeding a basal diet. Feed refusal during the five-day treatment period was evident in all treatment groups and was dose-related. There were statistically significant decreases in body weight gain during the five days of treatment in all groups except those birds fed a diet containing 200 mg/kg. No mortalities or overt signs of toxicity were observed at 200 or 560 mg/kg. Birds fed narasin at doses of 1800 and 5000 mg/kg feed appeared normal for the first four days. Lethargy and ataxia were observed in both groups on day five and deaths occurred on days five, six and eight. All surviving birds appeared normal at the termination of the study. The LD₅₀ at the end of the eight-day study was >5000 mg/kg. It was concluded that narasin did not have adverse effects on ducks at a dietary concentration of 200 mg/kg (data provided by industry).

4.3.4. Horses

The susceptibility of horses towards ionophoric compounds has been described in various studies. The results of a limited number of studies suggest that narasin is less toxic to horses than other ionophoric compounds, such as monensin. Two horses which received narasin intravenously at a single dose of 0.035 mg/kg b.w., showed neither remarkable changes in electrocardiographic parameters, haematology, blood chemistry, nor pathologic alterations during post-mortem inspection. However, one of the two horses showed ataxia, laboured respiration and sweating. Post-mortem investigations did not reveal significant pathological alterations (data provided by industry).

In the same study, no adverse clinical effects were observed when narasin was given by oral gavage at a dose of 0.8 mg /kg b.w. In contrast, following an oral gavage dose of 1.6 mg narasin/kg b.w., clinical signs included anorexia, uneasiness, ataxia, sweating, increased and laboured respiration, weakness, and recumbence. Both treated horses developed tachycardia, and atrial fibrillation occurred in one of the horses. Three days after oral administration of 1.6 mg narasin/kg b.w., the horses became moribund and at necropsy, pale areas in the heart, and reddening of the gastric

mucosa were observed. Histologically, slight focal degeneration and necroses were seen in the myocardium in both horses. Moreover, an increase in various serum enzyme levels was measured.

In addition, two feeding trials were performed in horses (data provided by industry). In the first experiment, group of horses (mares and geldings of mixed breeding; weight ranging from 391 to 536 kg), were given pelleted feed containing narasin at levels of 0, 80, or 240 mg/kg feed for one month. All three horses fed 240 mg/kg were recumbent after 24 hours and, after 48 hours, one horse was found dead, and the other two were moribund and had to be killed. None of the three horses fed 80 mg/kg died, but feed intake was reduced. In the second feeding trial, eight survivors from the first study were allotted randomly to individual pens after a washout period of two to three weeks. Four mares were fed narasin at a concentration of 400 mg/kg feed for 11 days. All horses were alive at the end of the study, except for one treated mare that was recumbent on day three, was moribund on day four and had to be humanely killed. Treatment-related gross lesions were found only in the mare that had been found moribund and consisted of pale foci and streaks on the heart, slight mottling of the liver, and few hemorrhagic areas in the stomach. Histologically, slight focal degeneration and necrosis of cardiac and skeletal muscles, and centrilobular degeneration and necrosis in the liver were found. The three other treated horses showed only minimal alterations in the striated muscle alterations and some evidence of skeletal muscle regeneration in one horse. From these feeding trials, it can be concluded that dietary concentrations of 240 mg narasin/kg feed or more are potentially lethal to horses. An oral dose of narasin that did not cause any adverse effects in horses was not identified, although the effects at 80 mg narasin/kg feed were restricted to reduced feed intake.

4.3.5. Cattle

In a repeated-dose toxicity trial, five Hereford heifers were allotted to five treatment groups (one animal per group). Narasin was administered by gavage at daily doses of 0, 0.5, 2, 4, or 8 mg/kg b.w. for 14 days. Animals administered doses of 4 and 8 mg/kg b.w. per day died either on day five or six respectively. Heifers that had received the lower doses survived the 14-day treatment with narasin. Narasin toxicity was associated with a dose-dependent decrease in feed consumption and weight gain. Gross pathologic examination revealed marked congestion and oedema of the lungs, and focal haemorrhages in the heart. Both animals from the 2 mg/kg b.w. per day group had focal degeneration and regeneration of skeletal muscle, and one had an enlarged heart with focal myocardial degeneration (data provided by industry). No clinical signs of toxicity were observed following the application of the low dose of 0.5 mg/kg b.w. per day (equivalent to 25 mg narasin/kg feed).

4.3.6. Pigs

Two studies were conducted to establish the safety of feeding narasin to growing and fattening pigs (data provided by industry). In the first study, 84 castrated male and 84 female pigs were allotted to a total of 7 pigs/pen and 12 pens/sex. Groups were fed diets containing narasin at 0, 25, 75, or 125 mg/kg, for 69 - 82 days. In the second study, four groups of three female and three castrated male pigs were fed with narasin at 0, 30, 45, or 60 mg/kg for 63 - 65 days. The measured endpoints included clinical signs and mortality, body weight, food consumption, and gross pathology. Clinical signs of toxicity were observed at the highest doses (75 and 125 mg/kg feed) in the first study for the first 8 to 14 days of treatment. Anorexia, dyspnoea, depression, leg weakness, ataxia, and recumbency were transient and did not reappear after 2 weeks of treatment. At these dietary levels, growth performance parameters declined in a dose-related manner; however, no adverse effects occurred in pigs fed with narasin at 25 mg/kg feed (first study) or in pigs receiving narasin at 30, 45 or 60 mg/kg feed (second study).

4.3.7. Rabbits

Groups of 3 male rabbits each received narasin at single oral gavage doses of 0, 10, 30, or 100 mg/kg b.w. (Novilla *et al.*, 1994). In animals given 30 mg/kg b.w. or higher, decreased locomotor activity, weakness in the extremities, and ataxia were observed at 3 hours after administration. In addition, relaxation of the abdominal muscle, prone position, ptosis, decreased respiration, and unusual breathing were present. Decreased locomotor activity, relaxation of abdominal muscle, prone position, ptosis, and unusual breathing were still observed 6 hours after narasin administration but the signs of weakness in extremities and ataxia were not seen at the 6 hour time point. No effects were seen at 10 mg/kg b.w.

Narasin poisoning in nine Hungarian rabbit warrens has been reported (Margit *et al.*, 1988). The clinical symptoms started with a significant decrease of feed intake, followed by uncoordinated movement, weakness and flaccid paralysis of the extremities, especially in the posterior body half. Nervous symptoms (tonic-clonic convulsions, affecting the entire body, as well as torticollis) were also observed. Death occurred one to four days after the onset of the clinical symptoms, frequently accompanied by significant malnutrition. In some acute cases, sudden deaths were also observed without any clinical signs. Enteritis and signs of circulatory disturbances were found in many cases. The histological lesions were characterised by moderate to severe Zenker's myofibrillar degeneration with lympho-histiocytic infiltration in the myocardium and skeletal musculature. Increases in serum enzyme activities (creatine-kinase, aspartate aminotransferase and alanine transaminase) showed a positive correlation with the morphological damage of the muscle tissues. Narasin concentrations varying between 35 and 150 mg/kg of feed caused acute intoxications and death.

4.3.8. Dogs

Although dogs are not food producing animals, incidental intoxications caused by ionophoric compounds have been observed in the past. In controlled toxicity studies the oral NOAEL was 0.5 mg/kg b.w., as neurotoxicity and skeletal muscle degeneration occurred at all higher doses in a 1-year study, suggesting that dogs are among the most sensitive animal species (Novilla *et al.*, 1994; EFSA, 2004).

No case reports of accidental dietary intoxications of dogs by narasin are available.

4.3.9. Fish

There are limited data on oral toxicity in fish. The 96 hours LC₅₀ for rainbow trout is 2.23 mg/L (EFSA, 2004).

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

4.4 Common drug-drug interaction

As for other ionophoric compounds, an interaction between narasin and pleuromutilins, such as tiamulin can not be excluded because it has been reported for other ionophores (Rátz *et al.*, 1997; Szucs *et al.*, 2004). However, none of the reports described the degree of adverse reactions that were caused by concurrent exposure to pleuromutilins and narasin.

5. Kinetics and tissue distribution

The kinetics of narasin have been described in the FEEDAP opinion (EFSA, 2004). Overall, the compound is rapidly metabolised and eliminated within a few days in most species (EFSA, 2004).

5.1. Kinetics of narasin in target animal species

5.1.1 Chickens

Chickens for fattening, which are the only target animal species for the use of narasin, received a single oral capsule of ¹⁴C-labelled narasin (80 mg narasin/kg b.w.). All animals showed a rapid excretion of the compound, with 85 % of the dose being detected in the excreta within 48 hours (EFSA, 2004).

Chickens (10 males and 10 females) receiving ^{14}C -labelled narasin supplemented feed at a concentration of 71 mg narasin/kg feed for five consecutive days, were slaughtered after a 6 hours withdrawal period. Narasin and its metabolites were extracted from the liver (49 % of the total radioactivity) and excreta (96 %). Identification of the labelled compounds revealed that one dihydroxy- and two trihydroxy-narasins were present in the liver and excreta. Parent narasin represented less than 1 % of the total radioactivity in the excreta, while three 9-ketotrihydroxy narasin metabolites were found representing a total of 49 % of the radioactivity (the major one representing 34 %) (EFSA, 2004).

In one experiment, three male and two female chickens were allowed *ad libitum* access to feed containing 50 mg/kg ^{14}C -labelled narasin for five consecutive days up to the time of slaughter (Sweeney *et al.*, 1996). Samples of leg and breast muscle (composite), abdominal fat, skin with attached subcutaneous fat, bile, the entire liver, and both kidneys were collected. The mean residue concentrations of narasin equivalents (total radioactive residues) were 0.32 mg/kg in liver, 0.12 mg/kg in fat, 0.08 mg/kg in skin/fat, 0.04 mg/kg in kidney, and <0.04 mg/kg in muscle. The radioactivity in fat was characterized as being predominately parent narasin (61 % of the fraction found in fat). Fifteen metabolites and the parent narasin were isolated from the excreta of chickens. These metabolites were predominately di- and trihydroxylated narasin metabolites and di- and trihydroxylated narasin B. These hydroxylated metabolites represented almost 50 % of the total radioactivity in the excreta, and only 3.0 % of the total radioactivity corresponded to narasin. The chromatographic distribution and relative magnitude of radioactivity from liver and excreta were similar, suggesting that excreta metabolites are the same as those found in liver (Sweeney *et al.*, 1996).

In another experiment, four male and four female chickens were fed with a commercial formulation of narasin (Monteban G100) (80 mg narasin/kg feed) for five consecutive days and then were slaughtered for residue analyses at 0, 6, 12 and 24 hours after feed withdrawal. Narasin concentration in tissues was determined by a validated HPLC method with a LOQ of 0.025 mg/kg and a LOD of 0.01 mg/kg. At zero withdrawal time, residue levels were only observed in fat/skin, liver and kidney at 0.059, <0.04 and <0.025 mg/kg. Narasin disappeared very rapidly from these tissues with the exception of fat tissues, where it was still measurable after 6 hours withdrawal, and even after 12 hours in some animals (EFSA, 2004).

In summary, narasin was excreted rapidly by chickens (85 % within 48 hours). Hydroxylation was the main metabolic pathway for narasin in chickens leading to the formation of di- and tri-hydroxy narasins. Unchanged parent narasin was a minor component (up to 3 %) whereas the hydroxylated metabolites represented 50 % of the total radioactivity in the excreta. Narasin metabolites were qualitatively similar in liver and excreta but not in fat, in which parent narasin represented 61 % of the measurable residues. Narasin was not detectable in tissues after a six hour withdrawal period, with the exception of the skin/fat (until 12 hours).

5.2. Kinetics of narasin in non-target animal species

5.2.1. Laying hens

Mature Single Comb White Leghorn hens (1.5 kg) were injected via cardiac puncture with a single dose of ¹⁴C-labelled narasin equivalent to 0.41 mg/kg b.w. Hens were sacrificed on days 1, 7, 14 and 28 post injection. Blood samples were taken at 0.5, 1, 1.5, 2, 3, 6, 9, 12 and 18 hours after dosing and also at the time of sacrifice. Excreta and eggs were collected daily. At the appointed slaughter times, liver, kidney, heart, ovary, fat, skin, bile and muscle were collected. Approximately 80 % of the dose had cleared from the plasma before the first blood sample was taken (0.5 hours). Samples of plasma taken at 24 hours post injection had only trace amounts of label. Most of the radioactivity was excreted within 24 hours with 93.6 % being excreted within 12 days. Only 1.32 % of the administered dose was recovered in eggs. On day 1, the liver contained the greatest amount of label followed by ovary, fat and skin (40.4, 15.4, 13.1 and 1.6 µg/kg equivalents). No residues were found in kidney on day 1 and no residues were observed in any of the tissues by day 7 (Catherman *et al.*, 1991).

Laying hens were fed contaminated feed containing 2.5 mg/kg narasin for 21 days followed by a seven day withdrawal period, and hens in a control group were fed non-medicated feed. Eggs were collected during the trial on days 0, 3, 7, 14, 21, and after a withdrawal period of seven days. The concentration of narasin in yolks and egg whites was analysed by a LC-MS method with a detection capability (CC_β) of 0.9 µg/kg. In the yolks, the mean concentration of narasin was 6.3 µg/kg at day seven, 7.1 µg/kg at day 14, and thereafter, it increased up to 10.6 µg/kg at day 21. No residues were found in the egg whites (Rokka *et al.*, 2005). By linear extrapolation, a concentration of 6, 15 and 30 µg narasin equivalents/kg in yolks would be anticipated if the hens had been given feed cross-contaminated at a level of 2, 5 and 10 %, respectively. Other authors reported a very high concentration of narasin (250 µg/kg) in egg whites and 800 mg/kg in yolks from laying hens that had received narasin at a level of 70 mg/kg in feed (Kan and Petz, 2000)²³.

Groups of 12 laying hens were treated with feed containing 2 mg and 40 mg narasin/kg feed for 14 days. Residues were determined and deposition and depletion curves were generated. The plateau concentrations in eggs from 2 and 40 mg narasin/kg feed were 6 and 90 µg/kg, respectively, and 8 and 18 days withdrawal were needed to reduce the concentration to below the CC_α of 2 µg/kg (Mortier *et al.*, 2005c).

Cooking narasin contaminated eggs for 10 minutes did not cause a statistically significant decrease in the amount of narasin present (Rokka *et al.*, 2005).

²³ In contrast, a higher exposure may occur when narasin is accidentally or illegally given to laying hens at the maximum authorised concentration of 70 mg/kg feed. This high dose results in residues amounting to 250 µg/kg of narasin in the egg white, and 800 µg/kg in the yolk (Kan and Petz, 2000). In this instance, an adult consumer consuming 100 g egg per day (about 50 g of yolk and 33 g of egg white) would receive a dose of 48 µg/person per day of narasin (equivalent to 0.8 µg/kg b.w. per day for a 60 kg adult) from the egg. This level is still below the ADI of 5 µg/kg b.w.

5.2.2. Turkeys

No studies of the kinetics of narasin in turkeys were available.

5.2.3. Japanese quail hens

Japanese quail hens (135 g) were injected via cardiac puncture a single dose of ^{14}C -labelled narasin equivalent to 0.74 mg/kg b.w. Quails were killed on days 1, 7, 14 and 28 post-injection. Blood samples were taken at 0.5, 1, 1.5, 2, 3, 6, 9, 12 and 18 hours post injection and also at the time of sacrifice. Excreta and eggs were collected daily. At the appointed slaughter times, liver, kidney, heart, ovary, fat, skin, bile and muscle were collected. Approximately 92 % of the dose had cleared plasma before the first blood sample was taken (0.5 hours). Samples of plasma taken at 24 hours post-injection had only trace amounts of label. Most of the radioactivity was excreted within 24 hours with 76.7 % being excreted within 12 days. In eggs only 4.18 % of the administered radiolabel was recovered. On day one, the ovary contained the greatest amount of radiolabel followed by liver and fat (111.5, 34.4 and 3.9 μg narasin equivalents/kg). No residues were found in kidney or skin on day one and no residues were found in any of the tissues by day seven (Catherman *et al.*, 1991).

Quail metabolised ^{14}C -labelled narasin via similar pathways to those in chickens but the excretion seemed to be more rapid. More radiolabel was recovered in the eggs of quail (4.18 % of the dose) than in the eggs of laying hens (1.32 % of the dose).

5.2.4. Pigs

Three groups of four pigs were fed ^{14}C -labelled narasin for 7 days at different concentrations: 30 mg/kg feed with no withdrawal period, 30 mg/kg with a three-day withdrawal period, and 45 mg/kg again without a withdrawal period prior to slaughter. Of the recovered radioactivity 5 % was found in urine and 95 % in the faeces. As in chickens, the metabolites identified in liver, bile, and faeces were oxidative and hydroxylated metabolites of narasin. The metabolite that was present in liver at the highest concentration was dihydroxynarasin B, representing 6 % of the total radioactivity in the liver. Analysis of liver did not detect any parent narasin above the LOQ of 25 $\mu\text{g}/\text{kg}$. The pigs fed 45 mg narasin/kg feed contained 1480 μg narasin equivalents/kg in the liver and 100, 90, 50 and 20 μg narasin equivalents/kg in fat, kidney, skin/fat and muscle, respectively (data provided by industry). By linear extrapolation, a concentration of 46, 115 and 230 μg narasin 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. The values for fat, kidney, skin/fat and muscle at a level of 10 % cross-contamination would be 16, 14, 8 and 3 μg narasin equivalents/kg, respectively.

Male and female pigs were fed a ration containing ^{14}C -labelled narasin at a level equivalent to 37.5 mg/kg feed for 5 days. Pigs were killed at 0, 24, 48 or 72 hours after the last exposure. Liver was

the only tissue found to contain radioactivity at concentrations of 510, 440, 260 and 180 µg narasin equivalents/kg at 0, 24, 48 and 72 hours, respectively. Although measured at zero withdrawal, four of six livers contained no detectable residue of parent narasin and two contained traces below 5 µg/kg (data provided by industry).

Narasin (¹⁴C-labelled) was fed to 12 male and 12 female pigs at doses of 45 mg/kg feed for 14 days. Liver and muscle tissues were collected at 12 hours (practical zero withdrawal). the concentration of parent narasin in liver and muscle was below the LOQ of 25 µg/kg.

In summary, in pigs, narasin was rapidly and extensively excreted, primarily in the faeces (95 %). Prior to excretion, narasin was extensively metabolised by hydroxylation, yielding diverse hydroxylated metabolites. Liver contained the largest amount of radioactivity but the level of parent narasin was below the LOQ (0.025 mg/kg) after 12 hours in all tissues, including the liver.

5.2.5. Other non-target animal species

No kinetic studies are available for non-target animal species other than laying hens, turkeys, quails and pigs.

No information was available on the potential for narasin 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 tolerance studies and toxicological investigations in domestic animals indicate significant species differences in the susceptibility to narasin and a small margin of safety (approximately 1.4) between the maximum authorised dose and the minimum toxic dose for chickens for fattening. Severe and even lethal intoxication were observed particularly in turkeys at levels in feed that were lower than the maximum authorised content of narasin in complete feeds for chickens. In contrast, ducks seem to have a relative high tolerance to narasin (no adverse effects at 200 mg narasin/kg diet). In horses, which are generally known to be susceptible to ionophoric compounds, only mild clinical alterations were observed following the consumption of feeds containing 80 mg/kg narasin, although a NOAEL was not identified. In contrast, in rabbits, narasin concentrations as low as 35 mg/kg feed caused severe acute intoxications. Taken together with information on other ionophoric substances, these data demonstrate not only significant species differences in toxicity, but show also that animals have diverging (and largely unpredictable) susceptibility to individual ionophoric compounds.

In conclusion, accidental ingestion of feed intended for chickens containing the maximum authorised level of 70 mg narasin per kg feed comprises a health risk for several other animal species, including rabbits, turkeys, laying hens, dogs, and possibly cattle, pigs and horses.

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

The data available on the occurrence of narasin in feed materials for non-target animal species describe a level of contamination varying between 0.015 and 3.6 mg/kg feed, with the exception of one sample of pig feed that contained 45.3 mg/kg (pigs tolerate narasin levels up to 60 mg/kg feed).

In general, the measured levels of narasin are below the maximum level of 7 mg/kg feed that would result from a rate of cross-contamination of 10 %. At 7 mg/kg feed an average feed intake of 50 g/kg b.w. per day, applicable to most monogastric animal species, would lead to an intake of 0.35 mg/kg b.w., which is below the overall NOAEL of 0.5 mg/kg b.w. (from a one-year dog study). 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 %.

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

The available experimental kinetic data suggest that residues are confined mainly to liver tissue and eggs, as narasin is rapidly metabolised and excreted and hence residues are rapidly depleted from all other tissues. In a worst case scenario, the results of a kinetic study with radiolabelled narasin in pigs given a diet containing 45 mg narasin/kg show that liver could contain residues of up to 1480 µg narasin equivalents/kg tissue. Residues of parent compound remained below the LOQ of 25 µg/kg after 12 hours in all tissues. By linear extrapolation it can be calculated that a feed concentration of 7 mg/kg feed (resulting from 10 % cross-contamination) would lead to 230 µg narasin equivalents/kg liver tissue. Concentrations in fat, kidney, skin/fat and muscle from this study were low (see 5.2.4.).

Experimental data demonstrate that the amount of narasin in the egg yolk of laying hens that were given feed containing 2.5 mg/kg of narasin reach a concentration of 10.6 µg/kg after 21 days of treatment. No narasin was detected in egg whites at any time. From this study it can be estimated by linear extrapolation that the residues occurring in yolk following exposure to feed containing 7 mg/kg narasin (10 % cross-contamination) would be 30 µg narasin equivalents/kg corresponding to approximately 15 µg narasin equivalents/kg in the whole egg.

Narasin was not detected in the majority of more than 1000 egg samples from several EU countries with methodologies that are capable of detecting narasin at concentrations as low as 0.1 - 2 µg/kg. The maximum detected level was 11 µg/kg egg. These results indicate that the actual residue levels will almost always be considerably lower than the theoretical estimates based on kinetic data.

No data are available on the possible carry-over of narasin 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 narasin from cross-contaminated feed are 100 g of liver and 100 g of eggs²⁴.

At the predicted concentration of narasin equivalents in pig liver resulting from 10 % cross-contamination (230 µg narasin equivalents/kg, see above), such liver consumption would lead to exposure to 23 µg narasin equivalents per person per day (corresponding to 0.38 µg/kg b.w. per day for a 60 kg adult). Since kinetic studies indicate that the residues of the parent compound are below the LOQ (25 µg/kg) this predicted concentration is considered to be an overestimation.

In addition, if the 100 g egg contains 50 g yolk, consumption of eggs from hens fed a 10 % cross-contaminated diet would then lead to a maximum intake of 1.5 µg narasin/person per day (corresponding to 0.025 µg/kg b.w. per day for a 60 kg adult).

The above estimates are 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 narasin resulting from cross-contamination of feed is likely to be infrequent.

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

²⁴ Values for daily human food consumption, as defined in Directive No (EC) 2001/79 are for birds: 300 g muscle, 100 g liver, 10 g kidney (50 g for mammals), 90 g skin/fat in natural proportions (50 g for mammals) and 100 g eggs (and 1500 g milk). Values for mammals are given in parenthesis when they differ from bird values

CONCLUSIONS

- Narasin exerts signs of toxicity typical of ionophoric compounds in various non-target animal species. Intoxications can be fatal, and may occur in sensitive animal species, for example in turkeys and rabbits, at feed concentrations below the maximum level authorised for use in chickens for fattening (70 mg narasin/kg feed).
- Cross-contamination of feed up to a level of 10 % is unlikely to comprise a significant risk for non-target animal species. This conclusion is based on model calculations of the potential exposure of animals and the comparison with the available toxicological data.
- The available data indicate that narasin residues would primarily occur in the liver and in eggs.
- Calculations based on the experimental kinetic data indicate that the maximum level of human exposure from narasin residues (parent compound and/or its metabolites) would be 0.38 and 0.025 µg/kg b.w. per day from pig liver and eggs, respectively, if feed is cross-contaminated at a level of 10 %. The available surveys conducted by Member States indicate that the actual levels of narasin found in liver and eggs are lower.
- Human exposure estimated to result from consumption of food products from non-target animal species exposed to cross-contaminated diet is well below the ADI of 5 µg/kg b.w. Therefore, the Panel concludes that there is negligible risk to consumers' health from the ingestion of narasin residues in animals exposed to feed cross-contaminated up to a level of 10 %.

DOCUMENTATION PROVIDED TO EFSA

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