

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

Scientific Opinion of the Panel on Contaminants in the Food Chain

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SUMMARY

Robenidine hydrochloride is a non-ionophoric synthetic compound that is authorised as a coccidiostat for use in chickens for fattening and turkeys at a minimum-maximum concentration of 30-36 mg/kg complete feed, and in rabbits for fattening at a minimum-maximum concentration of 50-66 mg/kg complete feed withdrawal period of 5 days for all target animals (Commission Regulation (EC) No 1800/2004²). Despite the requirements set for feed business operators in Regulation No (EC) 183/2005, it is generally acknowledged

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² OJ L 317, 16.10.2004, p. 37

that under practical conditions during the production of mixed feeds, a certain percentage of a feed batch remains in the production circuit and these residual amounts can contaminate the subsequent feed batches. This cross-contamination may result in the exposure of non-target animal species, and hence the potential health risks for non-target animal species as well as the potential residue deposition in foods derived from these non-target animal species have been evaluated.

Based on limited tolerance studies performed by industry on laying hens, pigs and ruminants, it is considered that accidental ingestion of feed intended for chickens, turkeys and rabbits containing robenidine at the maximum authorised level of 36, 36 and 66 mg/kg feed, respectively, does not present a health risk for these non-target animal species.

At a level of cross-contamination of 10% of the maximum authorised level, the intake of robenidine would be well below the overall no observed effect level (NOEL) of 7.5 mg/kg b.w. (based on liver enlargement derived from a 90 day dog study). Hence, the CONTAM Panel concluded that adverse effects are unlikely to occur in non-target animals as a result of cross-contamination of feed at a level up to 10% of the maximum authorised level of robenidine hydrochloride in feed for target animals.

No kinetic or occurrence data were available to estimate the amount of robenidine residues in milk, meat or offal from non-target animal species. Hence, consumer exposure was estimated using kinetic data from chickens for fattening fed the maximum level authorised for rabbits (66 mg robenidine/kg feed). These data were extrapolated to a concentration of 6.6 mg/kg feed to correspond to feed cross-contaminated with robenidine at a level of 10% of the maximum authorised level. Consumption of such poultry products (100 g of liver, 300 g muscle, 90 g skin/fat and 10 g kidney and 100 g eggs) could give an intake of 1.6 µg/kg b.w. for a 60 kg consumer, which represents 4.3% of the ADI of 37.5 µg/kg b.w. per day established by the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Therefore, even though kinetics and tissue deposition can differ between chickens for fattening and non-target animal species, the ADI is unlikely to be exceeded.

The CONTAM Panel concluded that the limited dataset provides no indication of an appreciable risk to consumers' health from the ingestion of robenidine residues in products from animals exposed to feed cross-contaminated up to a hypothetical level of 10% of the maximum authorised level for robenidine.

KEYWORDS: robenidine, cross-contamination, carry-over, coccidiostat, anticoccidial, 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) 95/69³

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 feedingstuffs containing premixtures prepared from coccidiostats have to receive approval for

³ 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)

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) 183/2005⁴

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 feedingstuffs⁵ 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

⁴ OJ L 35, 8.2.2005, p. 1

⁵ 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.

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

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

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

Coccidiostat (active substance)	Species or category of animals for which the use of coccidiostats is authorised (target animal)	Authorised maximum content of active substance in complete feed
Robenidine	Chickens for fattening Turkey Rabbits for fattening and breeding purposes	36 mg/kg (Cycostat) 36 mg/kg (Cycostat) 66 mg/kg (Cycostat)

4. Unavoidable cross-contamination (under practical conditions)

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

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

5. Tolerances

Therefore, the possibility to set tolerances for these unavoidable residues of coccidiostats in feedingstuffs for non-target species should be considered in the frame of Directive

2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed⁶.

Such tolerances for feedingstuffs for non target species could be set following the ALARA principle (As Low As Reasonably Achievable) taking into account good manufacturing practices. According to information received from professional organisations, a cross-contamination ranging in the area 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 species should not have a pharmacological activity and not endanger animal health and public health, as in some cases the tolerances for feedingstuffs for non target species could result in residues in products 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 robenidine 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 robenidine into the afterwards produced non target compound feed (for non target animal species).

The EFSA is requested to provide an opinion whereby

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

⁶ OJ L 140, 30.5.2002, p. 10. Directive as amended by Directive No 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 Council Directive 70/524/EEC and Council Regulation No (EC) 1831/2003 that repeals Directive 70/524/EEC (see also the background chapter). According to these provisions, authorisation and prerequisites for use of coccidiostats are defined for individual products (brands) following review by the EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) of data provided by the applicant.

Premixture: A mixture of feed additives with feed materials. Premixtures are not intended for direct consumption by animals, and are therefore not addressed in the Opinion.

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

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

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

ADI values: Acceptable daily intake (ADI) of a substance that can be consumed by a human over a lifetime without adverse health effects. As the CONTAM Panel did not have access to the complete safety (toxicological, pharmacological and microbiological) database available for the individual substances under consideration, the ADI value as derived by the FEEDAP Panel and where appropriate also the ADI(s) derived by other relevant scientific committees (e.g. the CVMP⁷ or the JECFA⁸) 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.

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) 1055/2006⁹ amending the Annexes I and III of Regulation No (EC) 2377/90¹⁰ to propose maximum residue limits (MRLs) for a number of coccidiostats. The FEEDAP has also recommended MRLs for some coccidiostats, and the CONTAM Panel considered these in the evaluation process.

Residues of coccidiostats in edible tissues, milk and eggs: According to Directive No (EC) 96/23¹¹ 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 ¹⁴C-radiolabelled, the concentration of total radioactive residue levels measured in the different tissues are expressed as µg parent coccidiostat equivalents/kg tissue, to indicate that these levels could be the parent compound and/or metabolites.

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

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

ASSESSMENT

1. Introduction

Robenidine hydrochloride is a non-ionophoric chemically synthesized substance. The chemical structure is presented in Figure 1.

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

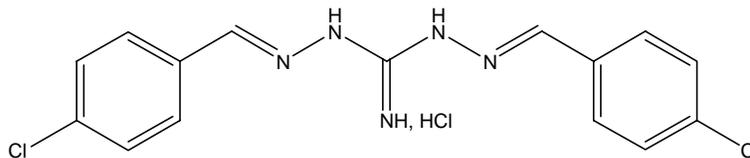


Figure 1. Chemical structure of robenidine hydrochloride

Robenidine hydrochloride [N¹,N³-bis [(p-chlorobenzylidene) amino] guanidine hydrochloride has the CAS number 25875-50-7, the molecular formula C₁₅H₁₃Cl₂N₅.HCl and the molecular weight of 370.7. The melting point is 288-290°C. It possesses a weak solubility in water (<1 mg/L), but is soluble in organic solvents: 94 mg/L in dimethylsulfoxide, 46 mg/L in dimethylformamide, 20 mg/L in pyridine and 6.3 mg/L in ethanol (95%). The Log Kow is 3.3 (EFSA, 2004a).

As summarised in the background chapter, robenidine hydrochloride in the form of a premixture with 66 g/kg carrier (Cycostat 66G) is authorised for use as a coccidiostat in chickens for fattening and turkeys at minimum-maximum concentrations of 30-36 mg/kg complete feed, and in rabbits for fattening at minimum-maximum concentration of 50-66 mg/kg complete feed (Regulation (EC) No 1800/2004¹²). The withdrawal period is five days in all species. Maximum residue limits (MRLs) have not been set for robenidine as yet.

1.1. Biological activities of robenidine

Anticoccidial activity

Robenidine hydrochloride activity against *Eimeria* species results from a dual action exerted upon different stages of the parasite as it develops in the intestinal mucosa (Ryley and Wilson, 1971). Initially it acts as a coccidiostat and arrests the development of the first schizont generation. Secondly, robenidine hydrochloride is coccidiocidal, killing the second generation of schizonts and possibly the merozoites. An additional effect against the sexual stage of coccidia development has been observed as well. Robenidine hydrochloride has been shown to be an inhibitor of oxidative phosphorylation (Wong *et al.*, 1972) and is reported to affect the protein metabolism and some other metabolic process of coccidia (Lee and Millard, 1972).

Antibacterial activity

Antibacterial activity (MIC) of robenidine has been assessed for reference strains and bacterial genera isolated from poultry and rabbits. The MIC values for Enterococci and Staphylococci varied between 8 and 16 µg/mL, for Clostridia between 1 and 8 µg/mL, and for Streptococci and Lactobacilli between 8 and 32 µg/mL. Robenidine had little or no activity against the Gram-negative bacteria tested (MIC>256 µg/mL). No relevant data are available

¹²OJ L 317, 16.10.2004, p. 37

on the development of resistance, cross-resistance and on effects on the colonisation of enteric pathogens such as *Salmonella* (EFSA, 2004a).

1.2. Previous evaluations of the toxicological properties and the safety of robenidine

The safety of robenidine has been assessed by the FEEDAP Panel, which considered the use of the robenidine-containing product Cycostat 66G as an additive to the feed of chickens for fattening, turkeys and rabbits. The FEEDAP Panel established an ADI for robenidine of 0.0375 mg/kg b.w. by applying an uncertainty factor of 200 to a NOEL of 7.5 mg/kg b.w. per day, based on liver enlargement in a 90 day dog study. An additional uncertainty factor of 2 was incorporated to account for the limited quality of the available studies. At higher doses (32.5 mg/kg b.w. per day) diffuse cytoplasmic vacuoles were observed in hepatocytes. Other toxicological studies that were considered included 90-day feeding studies in mice and rats, an 84-week feeding study in rats, a two-generation reproduction study in rats (incorporating an investigation of developmental toxicity), and a developmental toxicity study in rabbits. Mutagenicity tests (a bacterial reverse mutation test, an *in vitro* test for chromosomal aberrations in mammalian cells and a mouse bone marrow micronucleus test) gave uniformly negative results. No carcinogenicity study was provided, but limited histopathological examinations of tissues from the 84-week rat study showed no evidence of any treatment-related effect on tumour incidences (EFSA, 2004a,b).

1.3. Cross-contamination of feed batches

Feed additives, such as coccidiostats, are marketed as premixtures, intended to be incorporated into mixed feeds during the mixing and production process. Cross-contamination refers to the fact that under the practical conditions in a commercial feed mill, residual amounts of feed materials remain in the production line (see also the background chapter) and may contaminate following 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 the physicochemical characteristics of the product act together to determine the residual amount remaining in the circuit and hence the rate of cross-contamination from one feed batch to the subsequent batches produced in the same production line (Kennedy *et al.*, 1996, 1998b; Mc Evoy *et al.*, 2003; Harner *et al.*, 1996). In a similar

way, the purchased premix that is incorporated into the feed can itself contain traces of contamination of other substances, due to cross-contamination during the production of the premix.

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

- Areas of reduced flow in piping, material ledges, and non-plane surfaces (screw couplings, weld seams, moulded tanks) can lead to a sedimentation of feed materials.
- Oversized and long conveying systems, and non-continuous earthing of parts of the production plant.
- In silos or containers, differences in flow rate may cause segregation of the bulk material, which accumulates in dead zones with solidification of the bulk material.
- Conveyors which do not empty completely, such as screw conveyors and elevator boots.
- Wear of mixing equipment and conveying systems can cause a reduced flow in certain areas at which material can accumulate.
- Filter systems may accumulate residues, in particular with material featuring high dusting potential and strong aspiration flow.

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

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

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

occur at the feed plant during conveying (contaminated conveying equipment) and on-farm (e.g. during storage and transport to the feeding location).

1.3.2. Assessing cross-contamination in feed mills

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

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

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

1.4. Specific data for robenidine

The physical properties and the risk of feed contamination of 6.6% robenidine was assessed and compared to 6 other compounds. Tube residues (“tube active residue”) and dustiness (Heubach pulverimeter) was determined. Overall, cross-contamination with Robenidine 6.6 (1.57 mg/500g) was lower than that of nicarbazin 25% (1.8 mg/500g). Granulation of the active compounds is a common measure to decrease the risk of cross-contamination. Robenidine is produced and administered to feed as a granulate (Cycostat®). Experimental data showed that even the first mixed feed produced after the use of granulated additives (on a total of 5 subsequent mixing) did not contain detectable robenidine. Taking into account the above data it could be concluded that risk of contamination with Cycostat 66G (granulated Robenidine) is low (Jones *et al.*, 1990; Ascor Chimici srl, www.ascorchimici.it/eng/Granul_info_tec.asp).

2. Methods of analyses of robenidine

2.1. Analysis of robenidine in premixes and animal feeds

A method of analysis for the official control of robenidine in feedingstuffs has been established in EU legislation¹³. This method makes it possible to determine by reversed-phase high-performance liquid chromatography (HPLC) using an UV detector the levels of robenidine in feedingstuffs with a limit of quantification of 5 mg/kg.

In the process of approval of a feed additive containing robenidine the applicant presented a validated HPLC method for quantification of robenidine in premixes and feed (EFSA, 2004a). To detect a cross-contamination of 1% of the maximum authorised dose for a target animal species the method should be validated to a LOQ of 0.66 mg robenidine/kg feed. This method had a LOQ of 0.5 mg/kg feed and therefore meets the sensitivity requirements needed to monitor cross-contamination of robenidine in feed.

Another method was described by Mortier *et al.* (2005a). Robenidine samples were extracted with an organic solvent and analysed with a LC-MS/MS system. Detection was performed using multiple reaction monitoring (MRM) mode and electrospray ionisation. The method was fully validated and the regression analysis demonstrated a mean slope of 0.12, (std ± 0.002), a R^2 -value of 0.978 ($n = 11$) at the concentration range 0-2 mg/kg feed. The decision limit ($CC\alpha$)¹⁴ and the detection capability ($CC\beta$) was 0.0088 and 0.0125 mg/kg feed respectively.

2.2. Analysis of residue of robenidine in animal products

According to the list of methods used by the National Reference Laboratories (NRL) for residue control, edited by the Community Reference Laboratory (CRL), a robenidine method is reported for meat and eggs by 2 of the 20 NRLs within the EU. The method is based on liquid chromatography – tandem mass spectrometry (LC-MS/MS) and can be applied for screening and confirmatory purposes. Based on this method, decision limits of 1 μg robenidine/kg for both liver tissues and eggs were calculated (Bohm *et al.*, 2005; Daeseleire *et al.* 2005).

¹³ Directive 93/117/EEC of 17 December 1993 establishing Community analysis methods for official control of feedingstuffs (OJ L 329, 30.12.1993, p. 54-62)

¹⁴ Definitions of limit of detection (LOD), limit of quantification (LOQ), decision limit ($CC\alpha$) and detection capability ($CC\beta$): Commission decision 2002/657 of 12 August 2002 implementing Directive No (EC) 96/23 concerning the performance of analytical methods and the interpretation of results (OJ L 221, 17.08.2002, p. 8-36) define the performance of analytical methods used for residue control and the interpretation of results. $CC\alpha$ means the limit at and above which it can be concluded with an error probability of 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).

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

2.2.1. Screening methods

Previously, Dubois and colleagues (2004) described a qualitative multi-residue method based on LC-MS/MS for determining nine coccidiostats in muscle and eggs. The method uses extraction with acetonitrile followed by a clean-up by solid-phase extraction (SPE). Two mass transitions (m/z 334.1→138.2, m/z 334.1→111.2) were monitored. For robenidine residue in muscle, extraction recovery was 56% and $CC\alpha$ was 0.2 $\mu\text{g}/\text{kg}$. The method is also applicable to eggs.

2.2.2. Quantitative and confirmatory methods

A multi-residue method based on LC-MS/MS was described by Mortier *et al.* (2003). After acetonitrile extraction, the extract was concentrated and filtered before analysis. Three mass transitions (m/z 334.5→137.8, m/z 334.5→129.0, m/z 334.5→110.9) were monitored. Robenidine recovery was 42% and $CC\alpha$ was 3 $\mu\text{g}/\text{kg}$.

A method has been developed to analyse for robenidine residue in eggs (Dowling, 2005) using liquid chromatography with ultraviolet detection (LC-UV) at 317 nm. Egg samples were extracted with acetonitrile and the sample extracts were defatted with hexane. The method was validated. The mean recoveries ranged between 79 and 105%. The decision limit, $CC\alpha$, and the detection capability, $CC\beta$, were 10 and 17 $\mu\text{g}/\text{kg}$, respectively.

A method based on LC-MS/MS with positive electrospray was developed for analysis of robenidine residue in eggs by Daeseleire *et al.* (2005). After liquid-liquid extraction with acetonitrile, the extract was injected for liquid chromatography with an entry of the flow in the mass spectrometer at the elution time to reduced detector pollution. Diclazuril-bis was used as internal standard. Two mass transitions (m/z 334.1→155.1, m/z 334.1→138.1) were monitored. The concentration range was 0-75 $\mu\text{g}/\text{kg}$. For eggs, the mean slope was 1.29 ± 0.03 ($r^2=0.999$). The $CC\alpha$ and $CC\beta$ were 1 and 1.2 $\mu\text{g}/\text{kg}$ respectively. Recoveries ranged from $100.6 \pm 3.4\%$ at 50 $\mu\text{g}/\text{kg}$ to $104.9 \pm 2.3\%$ at 5 $\mu\text{g}/\text{kg}$.

3. Occurrence of robenidine

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

Data on cross-contamination of feed are scarce. The Czech Republic reported the results of 254 analyses that were performed during 2006. None of the investigated samples contained robenidine above the LOD of 0.5 mg/kg.

No notifications from the Rapid Alert System for Food and Feed (RASFF)¹⁵ have been sent concerning feed containing robenidine between April 2002 and April 2006.

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

Residues of robenidine 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) 96/23¹⁶. 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/kg tissue as their non-compliant limits.

For robenidine, combined results of 2004 and 2005 show that out of a total of 3095 samples of different animal tissues, seven out of 102 samples of rabbit muscle and eight out of 1431 samples of eggs were found to be non-compliant. All rabbit liver samples were compliant (57). The LOD ranged from 0.3 to 100 µg/kg. No details are given of the concentrations of robenidine residues that were found in the non-compliant samples.

Belgium has provided individual data of 972 samples of muscle tissue from different animals and eggs that were analysed in 2005 and 2006. Three samples of rabbit and one sample of poultry were higher than the Belgian non-compliant limit of 10 µg/kg. In addition, nine samples from rabbit and one sample from each egg and poultry, contained concentrations of robenidine that were greater than the LOQ of 5 µg/kg but less than the Belgian non-compliant limit. The exact concentrations were not given (data provided to EFSA).

¹⁵For more information on the RASFF system: http://ec.europa.eu/food/food/rapidalert/index_en.htm

¹⁶Directive 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.

In an incidence study (Mortier *et al.*, 2005b) of residues of anticoccidials in eggs in European countries, robenidine could be detected in 4.4% of the 320 samples analysed. The maximum concentration was 12 µg/kg.

4. Toxicity of robenidine

4.1. Mechanism of toxicity

The mechanism of toxicity of robenidine is unknown in mammals.

4.2. Toxicity of robenidine in target animal species

4.2.1. Chickens

The single oral LD₅₀ was found to be 450 mg/kg body weight. Several studies in chickens for fattening were performed from hatching to 8 weeks of age. Average weight gain, feed consumption, feed conversion and mortality were measured at time 0 and at the end of experiments. The highest tested dose of 300 mg/kg feed (8 times the maximum authorised level), resulted in significantly depressed weight gain and increased feed conversion rate compared to untreated birds. No other adverse effects were observed. Gross pathology and histopathological investigations (liver, kidney, spleen, gonads, brain and muscles examined) did not show any substance-related alterations. The maximum daily dose (36 mg/kg feed) given to a chicken would correspond to approximately 4.5 mg/kg body weight (EFSA, 2004a).

4.2.2. Turkeys

Studies in turkeys at doses of 0, 750, 1500 and 3000 mg robenidine/kg feed for 14 days showed that at a concentration of 750 mg robenidine per kg feed, the weight gain was slightly decreased as compared to the control animals, but no mortality occurred. At 1500 mg/kg there was a marked reduction in weight gain and two of six birds in one of the studies died. In a second study, all ten turkeys that had been given 3000 mg/kg feed died within 14 days (EFSA, 2004a). This dose of 3000 mg/kg feed is approximately 83 times higher than the maximum authorised level given as additive (36 mg/kg feed).

4.2.3. Rabbits

Robenidine administered in feed for rabbits at 0, 60, 100, 100/60, 200 and 500 mg/kg feed for 48 days did not affect any of the parameters examined (weight gain, feed conversion rate, feed intake, mortality and *post mortem* pathological changes) (EFSA, 2004a).

4.3. Toxicity of robenidine in non-target animal species

4.3.1. Laying hens

Laying hens were given robenidine at a dose of 0 or 60 mg/kg in the diet for ten days (data provided by industry). After treatment, the animals were crossed over and treated again for ten days. Feed consumption, body weight, clinical effects, egg production and egg quality were monitored. Robenidine at this dose regime did not adversely affect feed consumption, weight maintenance, or egg production. Layers for breeding (and cocks) were fed diets containing robenidine at a concentration of 0 or 30 mg/kg (equivalent to approximately 3.75 mg/kg body weight) for 4 weeks (data provided by industry). Eggs were collected from one week after the start of the treatment, until the end of the treatment. Egg production and quality were similar in both groups. Hatchability was very high in both group as well with a slight decrease (3%) in the treated group. No toxicity was observed on the chicks.

Day-old chicks receiving a diet containing robenidine at a concentration of 0 or 33 mg/kg for 190 days (data provided by industry) did not reveal any significant alterations of the ovaries and testicles as shown by gross and histological examinations.

Four groups of White Leghorn breeders were fed from 1 to 10 days of age diets containing robenidine at a dose of 0, 33, 66 or 132 mg/kg (data provided by the industry). During the study, body weight, feed consumption, egg production, feed efficiency, fertility and hatchability were recorded. There were no treatment-related effects for any of these parameters. These findings were supported by a study in which robenidine was administered to Arbor Acres broiler-breeders of each sex at 33 mg/kg in the feed with the objective to assess the reproductive response (Jones *et al.*, 1990). No differences were observed between controls and animals receiving robenidine on egg production, egg weight, hatchability or shell quality.

White Leghorns were fed diets containing robenidine from 26 weeks of age onward for 10 days (data provided by the industry). Robenidine was administered at a dose of 33 mg/kg in the diet and did not affect egg production, weight, Haugh Units (viscosity index), shell thickness or yolk mottling.

An undesirable property of robenidine is the unpleasant taste it imparts on chicken meat, if not withdrawn for 5 days before slaughter. The taste is also conveyed to eggs of layers when fed at the maximum authorised dose of 66 mg/kg feed (Booth and McDonald, 1982).

4.3.2. Pigs

In an open field trial, group of 10 weaned pigs received robenidine at concentrations of 0 or 120 mg/kg of feed, for 28 days. No differences between the two groups on weight gain and feed consumption were noted (data provided by the industry).

4.3.3. Ruminants

Groups of 6 Hereford calves were given robenidine at a concentration of 0 or 60 mg/kg of diet for 28 days (data provided by industry). There was no difference between groups in weight gain, feed consumption or feed efficiency.

4.3.4. Fish

In rainbow trout (*Salmo gairdneri* *Oncorhynchus mykiss*) the LC₅₀ of robenidine was found to be below 1 mg/L (Canton and van Esch, 1976).

No studies on oral toxicity were identified.

5. Kinetics and tissue distribution

5.1. Kinetics of robenidine in target animal species

Recently, the FEEDAP Panel has evaluated the coccidiostat Cycostat 66G in accordance with article 9G of Directive No 70/524/EEC (EFSA, 2004). Different kinetic studies of the dossier in chickens, turkeys and rabbits have been evaluated and are reported below.

5.1.1. Chickens

Male and female chickens received ¹⁴C-labelled robenidine by gavage (capsule) twice a day for seven consecutive days, at a dose corresponding to 36 mg/kg feed, i.e. the maximum authorised dietary concentration. Plasma and excreta sampling was performed during the entire experimental period, including the withdrawal time. Tissues were sampled for analysis at days 0 (zero withdrawal time), 1, 3, 5 and 7 of withdrawal. Plasma radioactivity increased rapidly and reached a plateau after 3 days of robenidine administration. At metabolic equilibrium after 7 days, 94% of the administered radioactivity was excreted within 1 day of the last dose and 97% within two days. Unchanged robenidine represented 44% (male) and 34% (female) of the radioactivity excreted.

At zero-withdrawal time, unchanged robenidine was the major tissue residue and represented 20%, 25%, 47% and 60% of the whole residues in the liver, kidney, muscle and skin plus fat respectively. Additional investigation showed that up to nine metabolites were detectable, which each represented less than 10% of the total radioactive residues. One major non-identified metabolite was isolated from the muscle, which represented 15% of the total radioactive residues. Three major metabolites were separated in the skin plus fat and four in the liver and kidney, which represented each between 3 and 15%. All along the withdrawal

period (1 to 7 days), the highest residues (total radioactive residues) were found in the liver, and by decreasing order in the skin plus fat, kidney and muscle being at zero withdrawal, 2520, 890, 1370 and 170 µg robenidine equivalents/kg, respectively. Residue levels of unchanged robenidine measured after 1 day withdrawal time were 230, 130, 420 and <100 µg/kg in liver, kidney, skin/fat and muscle, respectively. After 3 day withdrawal, unchanged robenidine residue levels were 100 µg/kg in all the tissues (EFSA, 2004). From these data it can be estimated by linear extrapolation that concentrations of 462, 163, 251 and 31 µg robenidine equivalents/kg tissue in liver, skin/fat, kidney and muscle would be anticipated if chickens were given feed cross-contaminated at a level of 10% of the maximum dose authorised of 66 mg robenidine/kg feed for rabbits.

5.1.2. Turkeys

Male and female animals received ¹⁴C-labelled robenidine orally in capsules twice a day for seven consecutive days, at a dose corresponding to 36 mg/kg feed, i.e. the maximum authorised dietary concentration. Plasma and excreta samples were collected during the entire experimental period, including a 7-day withdrawal period. The animals were slaughtered at 0, 1, 3, 5 and 7-day withdrawal time and tissues sampled for analysis. The main results were the following (EFSA, 2004): plasma radioactivity increased rapidly and reached a plateau after one day robenidine administration. At the metabolic steady state, 76% of the radioactivity administered was excreted within 24 hours. The extractability of the radioactivity in the excreta and tissues (at zero-withdrawal time) was very similar to that measured in the chicken. Unchanged robenidine represented 52% (male) and 44% (female), respectively of the radioactivity excreted, while the 8 measurable metabolites represented each less than 10%. At zero-withdrawal time, unchanged robenidine was found in different organs, at levels of 9-22% (female and male), 8-2%, 9-13% and 23-40% of the whole residues in the liver, kidney, muscle and skin plus fat, respectively. Up to six metabolites were isolated and separated from the liver and kidney, representing each between 1 and 14% of the total radioactive residues. In muscle tissue, four metabolites were isolated. These metabolites could be separated but not identified. The major metabolite in muscle tissue represented between 13% (females) and 35% (males) of the total radioactive residues. Three metabolites were found in the skin/fat, which each represented between 1 and 15% of the total radioactive residues. No qualitative gender differences were observed. During the withdrawal period, the highest level of total radioactive residues were found in the liver, and by decreasing order in the skin plus fat, kidney and muscle, being at zero withdrawal, 1610, 570, 830 and 70 µg robenidine equivalents/kg, respectively. Residue levels of unchanged robenidine measured after 1 day withdrawal time were 130, <100, 200 and <100 µg/kg in liver, kidney, skin/fat and muscle, respectively. After 3 days withdrawal, unchanged robenidine residue levels were <100 µg/kg in all tissues (EFSA, 2004). In conclusion, if turkeys are exposed to feed cross-contaminated at a level of 10% of the maximum level authorised, i.e. 66 mg robenidine/kg feed for rabbits,

by applying linear extrapolation, concentrations of 295, 104, 152 and 13 µg robenidine equivalents/kg tissue in liver, skin/fat, kidney and muscle, respectively, would be expected.

5.1.3. Rabbits

In model experiments, male and female animals received ¹⁴C-labelled robenidine orally in capsules twice a day for seven consecutive days, at a dose corresponding to 66 mg/kg feed, i.e. the maximum authorised dose level. Plasma radioactivity increased rapidly and reached a plateau within 24 hours. At steady state, 94% of the radioactivity administered was excreted within 1 day of the last dose. Of this amount, about 80% were excreted through the faeces, and 17% with urine. Unchanged robenidine represented 72% (male) and 82% (female) of the radioactivity excreted in the faeces but was not detected as such in the urine. In faeces, three metabolites representing each less than 10% of the whole faeces radioactivity, were found. In the urine, 12 metabolites were separated of which one major metabolite (58% and 36% in the male and female) was identified on the basis of its chromatographic behaviour as p-chlorobenzoic acid. All the other urinary metabolites were below 10%, of which p-chlorohippuric acid represented 4%. At zero-withdrawal time, unchanged robenidine represented 2-12 and 2% (female and male) and approximately 3% of the entire residues in the liver and kidney, respectively, but was absent in samples of muscle and abdominal fat. Up to three metabolites were isolated from muscle tissue, of which p-chlorobenzoic acid was the major one, represented 18-22% of total radioactive residues, while an unidentified metabolite represented 4-13% respectively. In the liver, p-chlorobenzoic acid was the major metabolite (6 and 12% for the male and female, respectively) while up to six metabolites represented each less than 10% of the total radioactive residues. Very similar figures were found in the kidney. In the abdominal fat three metabolites were isolated and separated from which a major unidentified metabolite represented 60 and 36% (male and female) of the total radioactive residues, the other three being each below 10%. Residue levels of total radioactivity expressed as mg robenidine equivalent/kg tissue measured at zero withdrawal time were 740, 540, 200 and 60 µg/kg in liver, kidney, skin/fat and muscle, respectively. After 7 days withdrawal, total radioactive residue levels were 210, 120, 30 and 20 µg/kg in liver, kidney, skin/fat and muscle, respectively. (EFSA, 2004a).

5.2. Kinetics of robenidine in the non-target animal species

5.2.1. Laying hens

In the study of Mortier *et al.* (2005c), laying hens were dosed with several coccidiostats at either the maximum allowed levels in chickens reared for fattening, or at 5% of this level. For robenidine, 12 animals per group were dosed at 36 mg/kg feed or at 1.8 mg/kg of feed for 14 days. Eggs were collected from the start until 30 days after the end of treatment (day 44).

Eggs were analysed by LC-MS/MS with a decision limit ($CC\alpha$) of 1 $\mu\text{g}/\text{kg}$. Maximal excretion into eggs (steady state) occurred after approximately 1 week, with concentrations of 1.3 mg/kg robenidine in the eggs produced at this time. Following withdrawal, residue concentrations in eggs produced by that group became less than the $CC\alpha$ after 26 days. Trace amounts were present in eggs produced up to day 29 of the withdrawal period. For the lower dose (5% of the authorised dose level) a maximum residue concentration of 0.07 mg/kg was reached in the eggs after 9 days of exposure. No steady state was observed, and concentrations declined rapidly. No residual amounts of robenidine could be found in eggs on day 13 after the end of treatment.

By linear extrapolation from the results of this study, a concentration of 47, 119 and 238 μg robenidine/kg egg would be anticipated if laying hens were given feed cross-contaminated at a level of 2, 5, 10% of the maximum authorised level of 66 mg robenidine/kg feed for rabbits.

5.2.2. Other non-target animal species

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

5.3. Common drug-drug interactions/incompatibilities

No interactions or incompatibilities of Cycostat 66G with feedingstuffs, carriers or other approved additives and veterinary medicinal products have been recorded or reported.

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

Based on a number of tolerance studies performed by industry on laying hens, pigs and ruminants, there is no evidence for the toxicity of robenidine in non-target animals exposed to the maximum authorised feed level for target animal species (see Chapter 4.2). There were also no case reports of intoxications under field conditions. These data suggest that an accidental ingestion of feed intended for chickens, turkeys and rabbits containing robenidine at the maximum authorised level of 36, 36 and 66 mg/kg feed, respectively, there is no indication of a health risk for common non-target animal species such as swine, cattle, goat and sheep.

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

In accordance with the Terms of Reference, levels of cross-contamination of 2, 5 and 10% of the maximum level authorised for rabbits (corresponding to 1.3, 3.3, or 6.6 mg robenidine per kg feed, respectively) were evaluated. At an average feed consumption of 50 g/kg b.w. in monogastric species, these concentrations would result in doses of approximately 0.066, 0.17 and 0.33 mg/kg b.w. per day, respectively. At a level of cross-contamination of 10% of the maximum level authorised for rabbits the intake of robenidine would be well below the overall NOEL (7.5 mg/kg b.w. per day derived from a 90 days dog study) (see Chapter 1.2). Therefore, the Panel concluded that adverse health effects in non-target animals are unlikely to result from cross-contamination of feed with robenidine up to a level of 10% of the maximum authorised level for rabbits and other target animal species.

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

In the limited surveillance data of residues of robenidine in food, occasionally non-compliant samples were reported. The actual concentrations found were not given (samples were denoted only as non-compliant) and therefore it is not possible to estimate the exposure to robenidine from residues in these non-compliant eggs and animal tissues. Moreover, no detailed kinetic studies in non-target animal species are available. Hence, as a general indication of the exposure, the CONTAM Panel decided to apply the data available from chickens for fattening to all non-target animal species, there being higher residues found in chickens than in other target species that were tested (turkeys and rabbits).

The kinetic data from chickens for fattening show that residues occur predominantly in liver tissues, whereas the residue levels in fat/skin, kidney and muscle tissues are lower. By linear extrapolation from the results for chickens given robenidine orally at doses equivalent to 36 mg/kg feed, a concentration of 462, 163, 251 and 31 µg robenidine equivalents/kg tissue in liver, skin/fat, kidney and muscle would be anticipated if chickens were given feed cross-contaminated at a level of 10% of the maximum authorised concentration of 66 mg robenidine/kg feed for rabbits.

Linear extrapolation from the results of kinetic studies in laying hens indicates that concentrations of 47, 119 and 238 µg robenidine/kg egg would be anticipated if laying hens were given feed cross-contaminated at a level of 2, 5, 10% of the maximum authorised level for rabbits of 66 mg robenidine/kg feed.

No data were available on the possible carry-over of robenidine 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

No occurrence data or kinetic studies were available to estimate the human exposure to robenidine residues in meat and organs from non-target animal species. Therefore, the exposure of consumers from consumption of foods, derived from animals exposed to cross-contaminated feed, was estimated from residues of robenidine in the liver, muscle, skin/fat and kidney of chickens for fattening that were given halofuginone by gavage at a dose corresponding to 36 mg/kg feed, extrapolated to a concentration of 6.6 mg/kg feed (10 % of the maximum authorised level for rabbits for fattening). The predicted concentrations of robenidine equivalents from a diet cross-contaminated with robenidine at 10% of the maximum authorised level were 462, 163, 251, 31 and 238 µg robenidine equivalents/kg tissue in liver, skin/fat, kidney, muscle and eggs, respectively. It was not possible to estimate the amount of robenidine in milk. The values for daily food consumption relevant for calculation of human exposure to robenidine from cross-contaminated feed are 100 g of liver, 10 g of kidney, 90 g skin/fat, 300 g muscle and 100 g eggs¹⁷. Such consumption would lead to an exposure of 96.5 µg robenidine equivalents/person per day (corresponding to 1.6 µg/kg b.w. per day for a 60 kg adult), which represents 4.3% of the ADI of 37.5 µg/kg b.w. per day. Even though the behaviour of the kinetic and tissue deposition can differ between chickens for fattening and non-target animal species, an exceedance of the ADI is unlikely to occur.

Therefore the Panel concluded that although the data provided was limited, there was no indication of an appreciable risk to the health of consumers from the ingestion of robenidine residues in products from animals exposed to feed cross-contaminated up to a hypothetical level of 10% of the maximum authorised level for rabbits and other target animals.

¹⁷ 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

- Robenidine has a low oral toxicity for target and non-target animals. The dog has been identified as the most sensitive species, and a NOEL of 7.5 mg/kg b.w. per day, based on liver enlargement, has been derived from a 90 day study.
- The limited tolerance studies with non-target animal species, including laying hens, pigs and ruminants, provide no evidence that accidental ingestion of feed intended for chickens, turkeys and rabbits, containing robenidine at the maximum authorised level of 36, 36 and 66 mg/kg feed, presents a health risk for non-target animal species.
- Animals exposed to cross-contaminated feed, containing robenidine at concentrations of up to 10% of the maximum authorised level, are unlikely to experience adverse health effects, as these concentrations are below the overall NOEL of 7.5 mg/kg b.w. as derived from a 90-day dog study. there was no indication of an appreciable risk to the health of consumers from the ingestion of robenidine residues in products
- No occurrence data or kinetic studies were available to estimate the human exposure to robenidine residues in milk or in meat and organs from non-target animal species. Therefore, the consumer exposure was estimated by extrapolating kinetic data from chickens for fattening. The human exposure from consumption of foods derived from non-target animal species exposed to 10% cross-contaminated diet was estimated to be 96.5 µg robenidine equivalents/person per day (corresponding to 1.6 µg/kg b.w. per day for a 60 kg adult), which represents 4.3% of the ADI of 37.5 µg/kg b.w.. Even though kinetic and tissue deposition may differ between chickens for fattening and non-target animal species, the ADI is unlikely to be exceeded.
- The Panel concluded that these limited datasets provided no indication of an appreciable risk to consumers' health from the ingestion of robenidine residues in products from animals exposed to feed cross-contaminated up to a hypothetical level of 10% of the maximum authorised dietary concentration for robenidine.

RECOMMENDATIONS

- Sensitive analytical methods are available for the detection of robenidine in animal products. These methods 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.
- In countries where robenidine is used, regular surveys for residues in animal products are recommended in order to evaluate human exposure to robenidine.

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Occurrence data

Belgium. AFSCA, The Food Agency.

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

European Commission, DG SANCO.