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REPORTS OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION

(Fourth series)

Report
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C O N T E N T S

FOREWORD

The fourth series of reports by the Scientific Cimmittee for Animal Nutrition (*) comprises opinions expressed by the Committee over the period 8 July 1981 to 1 June 1983 on the use of certain additives in feedingstuffs for poultry, pigs, cattle and rabbits. Most of these products, which are intended for promoting growth or preventing coccidiosis or histomoniasis, have either been authorized temporarily for use by Community legislation or the subject of an application for extension of use. Three products new for animal nutrition - one organic copper compound and two polyether compounds obtained from Streptomyces cultures - complete this series.

The rigorous assessments made by the Committee, particularly on metabolism, residues, microbiological and toxicological effects have clarified the optimum conditions of use of these additives and eliminated uncertainty about the safe use of a number of them. They have thus made an appreciable contribution to recent Community measures in the field of animal nutrition.

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(2) Appointed on 15/12/1981
(3) Up to 23/9/1982
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(9) Commission of the European Communities, Directorate General for Agriculture

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF OLAQUINDOX IN FEEDINGSTUFFS FOR PIGS

Opinion expressed 8 July 1981

TERMS OF REFERENCE (July 1978)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions:

1. Does the use of the growth promoter olaquindox in feedingstuffs for pigs, under the conditions of use authorized (see Background), result in the presence of residues in animal products? If so, what is the nature and the amount of these residues? Could these residues be harmful to the consumer?

2. Could the use of this additive affect the development of resistance in bacteria?

3. Could this use be prejudical to agricultural workers or to the environment? If so, what is the nature of the risks?

4. In the light of the answers to the above questions, should the conditions of use authorized for this additive be maintained or should they be modified?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the twenty-third Commission Directive of 23 June 1978 (2), Member States are authorized to use olaquindox, by way of derogation up to 31 December 1978, under the following conditions set out in Annex II, Section F, of the Directive:

Species of animal: pigs, up to four months.
Minimum and maximum content in complete feedingstuffs: 15-50 ppm (mg/kg); milk replacers: 50-100 ppm (mg/kg).
Other provisions: use prohibited for at least 4 weeks before slaughter. Mixing or simultaneous administration with an antibiotic prohibited.

OPINION OF THE COMMITTEE

1. When incorporated in feed for pigs, olaquindox [2-(N-2'-hydroxyethyl-carbonyl)-3-methylquinoxaline-1,4-dioxide] is absorbed from the digestive tract. The residues resulting from the use of the normal dose-level (50 mg/kg complete feedingstuff) for periods of eight weeks and from higher levels (100 and 160 mg/kg complete feedingstuff) for 20 weeks were determined in liver, kidney, muscle, adipose tissue and serum. In no case did the residues exceed the lower limit of determination (0.1 mg/kg by microbiological or spectrophometric analysis) 48 hours after the withdrawal of the supplemented feedingstuff.

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 198, 22.07.1978, p. 10

Investigations on the biotransformation using ^{14}C-labelled olaquindox showed that the residues consist of unchanged olaquindox and metabolites resulting from the reduction of the N=O radicals and from the oxydation of the alcohol group of the side chain. No trace of 2-carboxymethylaminocarbonyl-3-methylquinoxaline-1,4-dioxide was detected. Tissues taken 48 hours after oral administration of 2 mg labelled olaquindox/kg live weight contained residues of 0.01 to 0.07 mg/kg (by measurement of radioactivity expressed as olaquindox).

Olaquindox was investigated in short- and long-term toxicological studies in several species of laboratory animals. The levels without effect were evaluated at 1, 20 and 5 mg/kg live weight for rat, dog and monkey respectively. No teratogenic or carcinogenic effect was observed. A number of mutagenicity tests showed that the metabolite 2-carboxymethylaminocarbonyl-3-methylquinoxaline-1,4-dioxide, isolated from pig urine is mutagenic; the other metabolites are not mutagenic. The mutagenicity studies on olaquindox itself appear insufficient (Voogd et al., 1980). The Committee considers it necessary that extensive studies using a battery of tests covering not only bacterial test systems but also investigating other genetic endpoints in relation to chromosomal and DNA changes be carried out.

According to these data, residues resulting from the use of olaquindox in pig feed are below the acceptable limits and are no longer detectable after a four-week withdrawal period of the supplemented feedingstuff.

2. Olaquindox has antibacterial properties but does not lead to changes in the intestinal flora in pigs submitted to a diet containing up to 100 mg olaquindox/kg.

Investigations carried out over several years on more than 700 pigs showed that, at the normal level of use (50 mg/kg complete feeding-stuff), olaquindox does not lead to a selection of enterobacteriaceae carrying R-plasmids nor to a transfer of R-factors. It does not promote selection of intestinal bacteria resistant to tetracyclin, strepto- mycin, sulphafurazole, ampicillin or kanamycin nor does it favour the development of strains resistant to chloramphenicol. A slight reduc- tion in sensitivity of E. coli strains to olaquindox and in their rate of excretion in faeces was observed after a few weeks of treatment (Gedek 1979 a,b). The use of olaquindox as feed additive is thus without consequences for the use of antibiotics (in particular chloramphenicol) in human or veterinary therapy.

The Committee considers that the chemical and physico-chemical specifications of olaquindox and its preparations, presented in the dossier examined, are satisfactory. The Committee is not aware of undesirable effects arising during the handling of the product or its preparations. In the present state of knowledge, there is no evidence to suggest that a risk may be encountered by agricultural workers.

Olaquindox and its metabolites are excreted essentially in the urine and, to a small extent, in the faeces during the 48 hours following the administration to pigs of the supplemented feedingstuff. The products excreted in urine consist mainly of unchanged olaquindox and a monoxy-reduction product and, in smaller proportion, of derivatives of 2-carboxymethylaminocarbonyl-3-methylquinoxaline among which small amounts of the 1,4-dioxide are present.

The kinetics of degradation of olaquindox have been determined by measuring the antibacterial activity (E. coli). This compound is stable in aqueous solution but breaks down rapidly under the action of light. In liquid manures, biodegradation is practically complete within two to three days; in soils, it reaches 87 to 99% within 10 days. These observations indicate that accumulation in the environment is unlikely.

Olaquindox has a low toxicity (lethal concentrations : 1-10 mg/l) for protozoa, algae, daphnids, carps and eels and is not phytotoxic.

4. In the light of these data, the Committee is of the opinion that the use of olaquindox in feedingstuffs for pigs could be maintained provisionally in the conditions presently authorized. However, a reassessment of this additive is needed. For this purpose, the mutagenicity studies required by the Committee (see point 1 above) should be available.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF COPPER METHIONATE IN FEEDINGSTUFFS FOR PIGS

Opinion expressed 7 October 1981

TERMS OF REFERENCE (March 1980)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. What is the qualitative and quantitative composition of the residues in products of animal origin, arising from the use of copper methionate under the proposed conditions of use (see Backgorund)?

2. What is the qualitative and quantitative composition of the excreted products, deriving from this additive, under the proposed conditions of use?

3. Could the use of this additive result in biological or ecological effects different from those of the copper compounds already authorized as additives in feedingstuffs?

BACKGROUND

Copper methionate was the subject of an application for admission to Annex I, Section i of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs[1], under the following conditions of use:

Species of animal : pigs
Maximum content in complete feedingstuffs : 125 mg copper (total Cu)/kg.

(1) OJ No L 270 of 14.12.1970, p. 1

OPINION OF THE COMMITTEE

1. Copper methionate is a stable complex (chelate) formed from one atom of copper and two molecules of methionine. It is practically insoluble in water and organic solvents. It is not possible to determine the amount of copper methionate in feedingstuffs, body tissues or excreta because the chelate is decomposed during analysis. Only the levels of copper and methionine can be determined.

   Studies with copper methionate in the diet of pigs suggest that it will behave similarly to copper sulphate. Under the proposed conditions of use, the level of copper in pig liver did not differ appreciably from that arising from the use of copper compounds already authorized as feed additives.

2. According to the information available, the amount of excreted copper derived from feeding copper methionate is similar to that arising from feeding permitted copper compounds. The copper ingested with the feed is largely excreted in the faeces and only a small amount is retained in the body.

3. Taking into account the similar behaviour of copper methionate and other copper compounds already authorized in feedingstuffs, there is no reason to assume that the use of copper methionate as feed additive should result in biological or ecological effects notably different from those of the other copper compounds.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF LINCOMYCIN AND SPIRAMYCIN IN FEEDINGSTUFFS

Opinion expressed 7 October 1981

TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition is requested to re-assess lincomycin and spiramycin and ascertain whether the distinguishing factors were such as to justify different decisions on their acceptability as additives in feedingstuffs.

BACKGROUND

The Scientific Committee for Animal Nutrition delivered on 8 December 1977 its opinion on the use of macrolides and related products in feedingstuffs (*). It declared itself in favour of the use of spiramycin and reserved its opinion on lincomycin, partly because of the inadequacy of the existing data on bacterial resistance. The additional documentation now available apparently necessitates a review of the conditions laid down by Community directives for the use of these products as additives in feedingstuffs (see table below) and to ensure that such use will not entail any harmful effects on human or animal health.

(*) Commission of the European Communities. Reports of the Scientific Committee for Animal Nutrition. First series (1979). Catalogue No CB-28-79-277.

Additive	Species of animal	Maximum age	Minimum content ppm (mg/kg) of complete feedingstuff	Maximum content ppm (mg/kg) of complete feedingstuff
Lincomycin (*)	Poultry, with the exception of ducks, geese and laying hens	10 weeks	2	10
Spiramycin	Turkeys	26 weeks	5	20
	Other poultry, with the exception of ducks, geese, laying hens, pigeons	16 weeks	5	20
	Pigs, calves, lambs and kids	6 months	5	20 (80)**
	Animal bred for fur	-	5	20
	Piglets *	4 months	5	50
	Calves, lambs and kids *	16 weeks	5	50

* Use authorized by derogation until 30 June 1979
** Milk feeds

OPINION OF THE COMMITTEE

The Committee examined the documentation available on lincomycin and spiramycin and was of the opinion that the data given below should be taken into consideration when replying to the Commission's question.

1. Mode of action and bacterial resistance

Lincomycin is a pyranoside. It differs chemically from erythromycin and oleandomycin, which are macrocyclic C_{14}-lactones and from spiramycin and tylosin, which are macrocyclic C_{16}-lactones.

Lincomycin differs in principle from all known antibiotics but has some similarities to the macrolides in its mode of antibiotic action (30, 32). Like the macrolides it inhibits protein synthesis of the bacterial cell as a result of similarities in the binding sites on the ribosomes. Parallel resistance to macrolides or peptolide antibiotics such as virginiamycin, which has been observed in Gram-positive cocci, is attributed to this binding analogy (31). This resistance does not extend to other antibiotics because their mechanism of action is different (15). Strains of Staphylococcus aureus showing parallel resistance to macrolides and peptolide antibiotics have occurred relatively rarely under practical conditions (13, 14, 35). When lincomycin was first introduced, the levels of resistant strains were of the order of 2-9% for staphylococci and 5-6% for streptococci (groups A and B, and Viridans) depending on the origin of the samples. Similar figures are reported for those bacilli covered by the spectrum of action of lincomycin (32). Investigations performed on man have shown that the present level of resistance to lincomycin does not deviate appreciably from the abovementioned values (8, 17, 18, 34, 36).

In Belgium, where lincomycin has been used for years both as therapeutic agent and feed additive in livestock, 12% of S. aureus strains were found to be resistant to lincomycin (34). In investigations conducted in Belgium and the Federal Republic of Germany on volunteers, no resistance to this antibiotic was shown in strains of S. aureus of the same phage-type as those isolated from hospitalized patients (34). This underlines that the transmission of genes is less common than was assumed hitherto (27, 39).

In investigations carried out in 1977, 1978 and 1979 on stock farms in the Federal Republic of Germany, pigs were fed daily a medicated feedingstuff containing 100 to 150 mg lincomycin in mixture with spectinomycin. This treatment was given either prophylactically or therapeutically against enteritis of infectious origin or mycoplasma pneumonia. Tests on pigs and piglets given this treatment showed that the levels of staphylococci in the nose-mouth cavity and the skin flora, that were resistant to lincomycin, macrolides and virginiamycin did not differ from those observed in the untreated animals (13, 14, 35).

According to other studies, colibacilli (E. coli) of the intestinal flora of the pig showed a slight sensitivity to spectinomycin used in mixture with lincomycin, up to three weeks after administration; the susceptibility of the strains to lincomycin was not affected (8, 38) and the excretion of salmonellae was not favoured (37).

The various aspects of bacterial resistance to spiramycin have been studied extensively. Recent research confirms that spiramycin (a macrocyclic C_{16}-lactone) does not have the properties of inductive resistance seen with erythromycin and oleandomycin (macrocyclic C_{14}-lactones). At the levels used in nutritional studies, spiramycin does not promote parallel resistance to macrolides or substances with a mechanism of action comparable to that of the macrolides and does not exert direct or indirect selection pressure on bacteria carrying R-plasmids (14). Spiramycin has also been shown neither to promote the colonization of the digestive tract of farm animals by salmonellae nor to modify their excretion time.

2. Toxicity and residues

Lincomycin is partially metabolised in the animal organism. Three metabolites with a much lower antibiotic activity than that of lincomycin have been detected but not identified structurally (8).

At normal levels of use of lincomycin in chicken and turkey feedingstuffs (5 to 10 ppm), residues in muscle, adipose tissues, skin, liver and kidney, on completion of treatment, are below the minimum detectable by microbiological methods (0.6 mg/kg). Test in chicken with ^{14}C-labelled molecules showed detectable residues (detection limit by radioactivity: 0.1 mg/kg) only in the liver and offal.

A series of toxicological studies has been carried out with lincomycin in laboratory animals. Most of these tests were of short duration; administration was by subcutaneous, instramuscular or intravenous injection.

The LD_{50} after oral administration is greater than 4g/kg bodyweight in the rat. Oral administration to dogs over three months of lincomycin in aqueous solution at levels of 30, 100 and 300 mg/kg bodyweight/day produced no significant clinical, haematological or histopathological effects and did not affect animal weight or feed conversion rate. Short-lived diarrhoea was observed in some rats receiving orally 600 and 1000 mg/kg bodyweight for 3 months.

In a 26 months oral feeding study using the first filial generation which was exposed in utero, rats were fed doses of 0.375, 0.75 and 1.50 mg/kg bodyweight/day of premix grade lincomycin as well as 1.5 and 100 mg/kg bodyweight/day of USP grade lincomycin. No adverse effects were noted except acute prostatities and seminal vesiculitis in male rats at the highest level of premix and USP grade lincomycin tested. The no-effect level has been estimated at 0.75 mg/kg bodyweight in the rat. From this an ADI of 0.0075 mg/kg bodyweight was determined. The results of a chronic feeding study in mice are not available.

A three-months study in dogs using oral administration revealed a significant but temporary increase in the serum glumatic-pyruvic transaminase level when lincomycin was tested at the dose-levels of 400 and 800 mg/kg bodyweight/day. A six-months study involving doses of 30, 100 and 300 mg/kg bodyweight/day revealed no clinical or haematological adverse effects and no influence on organ weights. A lymphocytic thyroiditis was observed in some animals at the dose of 300 mg/kg bodyweight. A one-year study in beagles used oral dose levels of 0.375, 0.75 and 1.5 mg/kg bodyweight of premix grade lincomycin as well as 1.5 mg and 100 mg/kg bodyweight of USP grade lincomycin. No abnormalities were noted.

A three-generation reproduction study in rats using dose levels of 0.375, 0.75 and 1.5 mg/kg bodyweight of premix grade lincomycin as well as 1.5 mg and 100 mg/kg bodyweight of USP grade lincomycin revealed no adverse effects.

A teratology study in rats using oral dose levels of 10, 30 and 100 mg/kg bodyweight on days 6 through 15 of gestation showed no teratogenic effects but there was increased embryo lethality at the 100 mg/kg bodyweight level.

Oral administration of a single 50 mg dose induces diarrhoea in the rabbit followed by death within four to eight days. The same effect has been observed in the guinea pig after subcutaneous injection of small doses of lincomycin. This was attributed to imbalance in the intestinal microbial flora.

Spiramycin is partially metabolized in the animal organism into neospiramycin and unidentified unstable polar derivatives (9).

At the normal levels of use of spiramycin in chicken and pig feedingstuffs (10-20 ppm), residue levels in the tissue, on completion of treatment, are generally below the minimum that can be determined microbiologically (limit of detection: 0.02 mg/kg). Residues of 0.02 to 0.8 mg/kg and of 0.02 to 0.06 mg/kg have been detected in the liver of chicken immediately after treatment and after a three day withdrawal period respectively. The residues were 0.18 to 0.31 mg/kg in the liver of pigs immediately after treatment and 0.17 to 0.18 mg/kg sixteen hours after administration has been stopped. The maximum residue level in the kidneys of chicken and pigs was 0.2 mg/kg immediately after treatment.

Spiramycin has been investigated in short- and long-term toxicological studies in several animal species. Oral administration to mice of a single dose of 5 mg/kg bodyweight did not cause death. A 2-year study

in rats given diets containing 1500, 3000 and 6000 mg spiramycin/kg feed revealed no adverse clinical, haematological, biochemical or histopathological effects and no carcinogenic activity. The no-effect level has been estimated at 75 mg/kg bodyweight for rats. The acceptable daily intake has been established at 0.75 mg/kg bodyweight.

Daily administration of spiramycin up to 350 mg/kg bodyweight in the feed of pregnant rats produced no teratogenic or embryotowic effects. The development of the foetuses and new-born animals was normal (40).

Advantages in animal husbandry

The recommended lincomycin content for poultry feedingstuffs is 2-10 g per tonne (2-10 ppm).

Fattening chickens showed average improvement of 2.7% in weight gain and 2.3% in feed conversion ratio in 31 experimental studies carried out under various stock rearing conditions (different animal strains, feed rations with different energy- and protein contents). These results have been confirmed in practice (21). The addition of lincomycin is said also to improve viability and to reduce morbidity in chickens.

The improvement obtained with spiramycin used at authorized dose levels in various animal species ranged in average from 4 to 7% in weight gain and from 2.5 to 5% in feed conversion ratio according to a number of studies. Experiments in official stations over the past 11 years in chicken and over the past 13 years in pigs have shown continued beneficial effects. It is therefore likely that the use of

spiramycin in nutritional doses favourably affects animal health without directly interfering with pathogenic bacteria; this is borne out by the fact that the product retains its full activity when used in therapeutic doses.

4. Therapeutic indications

Lincomycin is presently used for the treatment of infections due to staphylococci and to streptococci, except enterococci, especially where β-lactam antibiotics cannot be used. In addition infections caused by clostridia, corynebacteria or mycoplasma are likely to respond.

The main factors leading to the gradual restriction of the therapeutic applications of lincomycin in human medicine are as follows :

(a) Reduced absorption of the antibiotic when administered orally. It is therefore necessary to use relatively high doses with the consequent risk of causing an imbalance in the intestinal microbial flora (12, 25, 29).

(b) Lower antibacterial action on anaerobic intestinal bacteria than is shown by other antibiotics and semi-synthetic derivative of lincomycin (1, 4, 16, 22, 26, 28, 33).

(c) Fatal cases of enterocolitis occuring during therapeutic treatment as a result of imbalance in the intestinal microbial flora and possible endotoxin production (2, 3, 5, 6, 7, 10, 11, 19, 20, 23, 24).

The spectrum of antibacterial activity of spiramycin includes Gram-positive bacteria (except enterococci), neisseria and mycoplasma. Its therapeutic efficacy is limited because of its incomplete absorption and by the pH of the organic medium at the site of action. Spiramycin is suitable for use in dental medicine because of its high level of elimination in the saliva (32).

The recommended therapeutic indications are as follows. For human therapy : infections of the upper and lower respiratory tracts with Gram-positive cocci (dental infections, tonsillitis, rhinopharyngitis, otitis, bronchitis, acute lung infections, respiratory complications in eruptive diseases). In veterinary medicine : infections caused by Gram-positive cocci and mycoplasma (lung infections and infectious enteritis in cattle and pigs, mastitis, respiratory diseases in poultry).

Lincomycin and spiramycin consumption for therapeutic purposes in the Community accounts for only a very small percentage (2-3%) of all antibiotics used. Spiramycin consumption has remained relatively constant in recent years while lincomycin consumption has tended to decline.

In summary, the comparison of lincomycin with spyramycin shows :

- The two substances belong to different chemical groups but have a similar mode of antibiotic action.

- Their use in nutritional doses has no significant effect on bacterial resistance.

- Short-term and long-term toxicity studies have been conducted on both substances. A no-effect level and an acceptable daily intake have been established for each of them.

- The tissue residues of both substances are much below the acceptable limits in the conditions under which they are authorized for use as feed additives.

- When used as feed additives, both substances have shown demonstrable advantages in terms of animal husbandry.

- The therapeutic indications for the two substances in man and animals are specific. Their use is limited. Their consumption in the Community accounts for only 2-3% of all antibiotics used. There is nothing to indicate that the therapeutic efficacy of these two antibiotics is deleteriously affected by their use as additives in feedingstuffs.

In conclusion, the Committee expresses the following opinion:

1. Lincomycin

In the light of the information now available, the Committee is of the opinion that the use of this antibiotic as a feed additive is acceptable under the conditions authorized (see table p. 10).

2. Spiramycin

In the light of the information now available, the Committee confirms the favourable opinion it delivered in 1977 on the use of this antibiotic as a feed additive under the conditions authorized (see table p. 10).

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF MONENSIN SODIUM IN FEEDINGSTUFFS FOR POULTRY

Opinion expressed 11 March and 9 December 1981

TERMS OF REFERENCE (July 1980)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions:

A. Chickens for fattening

1. What is the qualitative and quantitative composition of the residues in tissues and organs of the chicken arising from the administration up to slaughter of 100-125 mg monensin sodium/kg complete feedingstuff?

2. Are these residues free of risks for the consumer?

3. In the light of the answers to the above questions, could the withdrawal period of the supplemented feedingstuffs, set at a minimum of three days, be reduced or abolished?

B. Chickens reared for laying

Can the use of monensin sodium at dose-levels of 100-120 mg/kg complete feedingstuff up to the age of 16 weeks for chickens reared for laying give rise to residues in eggs?

C. Turkeys

1. Does the use of monensin sodium under the conditions proposed for feedingstuffs for turkeys (see Background) result in the presence of residues in tissues and organs of the animal? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

2. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

3. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the thirty-third Commission Directive of 4 July 1980 (2), the use of monensin sodium as a coccidiostat is authorized at Community level under the conditions set out as follows :

Species of animal : chickens for fattening.
Minimum and maximum content in complete feedingstuffs : 100-125 ppm (mg/kg).
Other provisions : use prohibited at least three days before slaughter.

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 185, 18.07.1980, p. 48

The following proposals were presented :

(a) To delete the provision concerning the withdrawal period of the supplemented feedingstuff before slaughter.

(b) To extend the authorization of use of the product by the following provisions :

Species of animal : chickens reared for laying (up to 16 weeks), turkeys.
Minimum and maximum content in complete feedingstuffs : 100-120 ppm (mg/kg).
Other provisions : for turkeys, use prohibited at least three days before slaughter.

OPINION OF THE COMMITTEE

A. Chickens for fattening

1. Monensin sodium, when administered in feedingstuffs for chickens, is weakly absorbed from the digestive tract. The microbiological determination of residues in fat, skin, kidneys, muscle and liver of chickens fed for several weeks and without a withdrawal period on a ration supplemented with 121 mg monensin/kg has shown that 12% of the samples contained residues in amounts higher than 0.05 mg/kg but generally not exceeding 0.1 mg/kg. The presence of these residues was most frequent in fat samples.

The study of distribution, composition and elimination of monensin residues, performed with the ^{14}C-labelled product, has shown that, under the abovementioned conditions of use, the residues in liver and kidneys were higher and of different composition than those in fat. The greater proportion of monensin was found in fat (1/3 of the radioactivity). Residues in the liver consisted to a small extent of monensin but a large number of metabolites, among them demethylation, hydroxylation and decarboxylation derivatives similar to those isolated from bovine liver, were identified.

These residues disappear rapidly. After three days withdrawal of the supplemented feedingstuff, only the liver and kidneys still contained traces of residues detectable by radioactivity (limit of detection : 0.02-0.04 mg/kg, expressed as monensin sodium).

2. Short- and long-term toxicity studies have been carried out on monensin sodium. The no-effect level in rats was 1.25 mg/kg bodyweight.

   The small amounts of residues which can be present in edible tissues of chickens when the supplemented feedingstuff is administered up to slaughter are free of risks for the consumer.

3. Notwithstanding the absence of toxicological effects of the residues, the Committee is of the opinion that it would be prudent to maintain the withdrawal period of a least three days before slaughter.

B. Chickens reared for laying

Some of the absorbed monensin sodium passes into the eggs and can be microbiologically determined as long as the supplemented feed is not withdrawn before laying. Microbiological determination with Bacillus subtilis (limit of detection : 0.025 mg/kg) on eggs from various groups of laying hens which had received monensin at dose-levels of 132 mg (from the 1st to the 40th day), 110 mg (from the 41st to the 82nd day) and 88 mg (from the 83rd to the 104th day) per kg complete feedingstuff showed residues (0.025-0.05 mg/kg) until the 2nd or 3rd day after withdrawal of the supplemented feed. Eggs laid on the 4th and 5th days after withdrawal no longer contained residues detectable microbiologically. The qualitative and quantitative composition of possible metabolites is not known.

In controlled conditions, lighting and feeding regimes are applied to ensure that laying starts in about the 20th week; this improves the optimum size of the first eggs laid. However, under poor technical conditions and in countries where the sky is brighter, laying may start as early as in the 16th week because of stimulation of the reproductive tract.

Bearing in mind that the effectiveness of the anticoccidial treatment with monensin and the resulting immunity are greatest at the eighth week, the Committee recommends as a precautionary measure that at dose-levels of 100-120 mg/kg complete feedingstuff the product be used up to no longer than the 15th week. This limitation is adequate to ensure the absence of microbiologically detectable residues in eggs.

C. Turkeys

The Committee proposed to express its opinion when data on metabolism of monensin sodium, its residues and excreted products in turkeys become available.

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REPORT BY THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF ROBENIDINE IN FEEDINGSTUFFS FOR RABBITS

Opinion expressed 10 February 1982

TERMS OF REFERENCE (November 1980)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of the coccidiostat robenidine under the conditions proposed for feedingstuffs for rabbits (see Background) result in the presence of residues in tissues and organs of the animal? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful for the consumer?

2. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

3. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by the 34th Commission Directive of 4 September 1980 (2), the use of robenidine is authorized at Community level under the conditions laid down in Section D of Annex I to the Directive, namely :

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 251, 24.09.1980, p. 17

Species of animal : chickens for fattening, turkeys.
Minimum and maximum content in complete feedingstuffs : 30-36 ppm (mg/kg).
Other provisions : use prohibited at least five days before slaughter.

It is proposed that authorization of the use of this additive be extended to include the following provisions :

Species of animal : rabbits.
Minimum and maximum content in complete feedingstuffs : 50-66 ppm (mg/kg).

OPINION OF THE COMMITTEE

1. Studies of the metabolism of robenidine (1,3-bis [p-chlorobenzylidene amino]guanidine) in the rabbit, using molecules labelled with ^{14}C in the aromatic nucleus, indicate that the product is partially absorbed and that it is metabolized to p-chlorobenzoic acid by a process of hydrolysis and oxidation. The resulting p-chlorobenzoic acid combines partially with the amino acids to give more polar compounds such as p-chlorohippuric acid.

   95% of the total radioactivity is eliminated within 48 hours of administration, 16-18% in the urine. Tissue residues dissapear for the most part within 24 hours; small quantities persist for a few days. In the liver, measurement of radioactivity expressed as robenidine gave 0.7 and 0.4 mg/kg on day 5 and day 8 following administration per os of 0.37 mg of product/animal/day. Analyses showed that 85% of the radioactivity was due to the presence of p-chlorobenzoic acid and the product of conjugation of this acid with glycine (p-chlorohippuric acid), the remainder consisting of unidentified compounds, strongly bound to proteins.

Residues were also studies after administration of diets containing 55 and 67 mg of robenidine/kg complete feed for 7 and 12 weeks. No residue of robenidine was detected in liver, muscle, kidney or fat by high pressure liquid chromatography (detection limit : 0.1 mg/kg), even in case where the supplemented feed had been administered until slaughter.

Short- and long-term studies of the toxicity of robenidine have been carried out on several species of animal. The no-effect level is 100 mg/kg of feed in the mouse and 150-200 mg/kg of feed in the rat. At high doses, robenidine has toxic effects on the kidney, resulting in morphological changes (vacuolization of tubular cells). No carcinogenic effect was noted in either species of rodent (rat or mouse), nor any evidence of microbial mutagenesis. A study of reproduction in the rabbit and rat revealed no abnormalities, even in cases where doses ten times higher than the proposed dose were administered in the feed.

The proposed use therefore presents no risks to the consumer. A withdrawal period of no less than five days before slaughter is nonetheless proposed as a precaution to ensure the elimination of bioavailable residues.

2. Studies of the excretion of robenidine by the rabbit show that, in the proposed conditions of use, more than 70% of the ingested quantity is eliminated unchanged in faecal matter. The rest is eliminated mainly in the form of free or conjugated p-chlorobenzoic acid in the urine. Consequently, recycling by caecotrophy mainly concerns untransformed robenidine; it contributed to the reabsorption of about 8% of the robenidine present in the feedingstuff.

Biodegradation of robenidine in the environment has been studied with the aid of molecules labelled with ^{14}C (in the guanidine radical) in an ecosystem model consisting of a soil/water interphase comprising grasses (Sorghum halpense), algae (Rhizoclonium and Lyngbia Sp.), crustaceans (Daphnia magna), molluscs (Gyraulis Sp.), larvae of lepidoptera (Estigmene acrea), and mosquitos (Anopheles quadrimaculatus), and fish (Gambusia affinis). The labelled robenidine was incoporated in feedingstuffs for turkeys at the level of 66 mg/kg and the droppings of these birds, which contained 60% of the ingested, non-metabolized robenidine, were mixed with the upper layer of soil in the ecosystem.

The results of the study showed that robenidine, which is insoluble in water, is metabolized slowly in the soil into polar compounds whose identity has not been established. These polar compounds are either absorbed by the soil or carried away by water. After eighty days, the quantity of non-transformed robenidine in the soil was only 8% of the initial value; the quantity in the water was 0,13 µg/l.

Bioconcentration, i.e. the ratio between the concentration of ^{14}C in plant and animal species and the concentration of ^{14}C in the water of the ecosystem, was in all cases below the value observed in identical experimental conditions for DDT labelled with ^{14}C. Robenidine and its metabolites are not phytotoxic. They are relatively toxic for aquatic organisms, especially Daphnia magna (LC_{50} after 48 h : 56 µg/l) and Salmo gairdnerii (LC_{50} after 48 h: 75 µg/l). However, under natural conditions in water, it does not seem that toxic concentrations could be reached, even for sensitive species.

3. In the light of the available information, the Committee considers that the use of robenidine in feedingstuffs for rabbits, at use level of 50 to 66 ppm (mg/kg) is acceptable subject to a withdrawal period of not less than five days before slaughter.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITTION ON THE USE OF SALINOMYCIN IN FEEDINGSTUFFS

Opinion expressed 14 April 1982

TERMS OF REFERENCE (November 1980)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of salinomycin sodium in animal feedingstuffs, under the proposed conditions of use (see Background), result in the presence of residues in animal products? If so, what is the nature and the amount of these residues? Could these residues be harmful to the consumer?

2. Could the use of this additive affect the development of resistance in bacteria?

3. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

4. In the light of the answers to the above question, are the proposed conditions of use acceptable?

BACKGROUND

Salinomycin sodium was the subject of a submission for inclusion in Annex II of Council Directive 70/524/EEC of 23 November 1970, concerning additives in feedingstuffs (1), as a coccidiostat for fattening chickens and turkeys, and rabbits, and as a growth promoter (group of antibiotics)

(1) OJ No L 270 of 14.12.1970, p. 1

for piglets, pigs, lambs and fattening cattle, under the following conditions :

	Minimum content ppm (mg/kg) of complete	Maximum content feedingstuffs
Chickens for fattening *	50	70
Turkeys for fattening *	20	30
Rabbits *	25	35
Piglets, up to 16 weeks	50	80
Pigs, up to 6 months	25	50
Lambs	10	30
Cattle for fattening	10	30

* Use prohibited at least five days before slaughter.

OPINION OF THE COMMITTEE

1. Salinomycin is a polyether antibiotic of known structure. It is a monobasic carboxylic acid containing five cyclic ether rings. Metabolism studies in mice, rats and chicken using oral doses of ^{14}C-labelled salinomycin showed that over 90% is excreted in the faeces within 48-72 hours, less than 5% in the urine and negligible amounts in the expired air. Small amounts of ^{14}C-salinomycin are found in the liver and bile after 48-72 hours. Salinomycin is rapidly metabolized in the gut and liver to numerous metabolites, some of which are 5, 15-dihydroxy derivatives. Metabolism is slowest in chicken, where ^{14}C-labelled residues in the liver amount to 0.1-0.4 mg/kg after 120 hours. In cattle, orally administered ^{14}C-salinomycin, 90.5% appears in the faeces, 1.4% in the urine, 0.6% in the

liver and 4.2% in all other tissues (at levels of < 0.1 mg/kg) after 4 days. Pigs excrete on average 83.5% in the faeces, 2.1% in the urine, with < 0.1 mg/kg in the liver and < 0.01 mg/kg in muscle, kidney and fat remaining after 4 days (limit of detection of radiochemical method 0.01 mg/kg). No data using ^{14}C-salinomycin are available on sheep.

Extensive residue studies in broilers, turkeys, rabbits and pigs showed no detectable residues after 24 hours withdrawal using a microbiological method (limit of detection 0.01 mg/kg). The exact chemical nature of most residues has not been established. Residues in the liver of sheep and cattle after 24 hours ranged from 0.06-0.17 mg/kg and had disappeared after 3-5 days (limit of detection by microbiological method 0.01 mg/kg). When fed erroneously to laying hens, dose-related residues appear in the yolk of eggs ranging from 0.2-0.3 mg/kg within 3 days and disappearing, if 5 days withdrawal is interposed.

Acute toxicity studies with dried mycelium, with the product without mycelium, or with pure substance in mice, rats, chicken, rabbits, dogs, pigs, bulls and horses showed oral LD_{50} values from 60-21 mg/kg b.w.; for mice, rats, chicken and rabbits the signs of toxicity being mostly neurological. Pigs, bulls and horses were increasingly sensitive in that order, toxic effects occuring mostly in the liver and myocardium. Subchronic studies were carried out in mice, rats, dogs and pigs. The target organs of toxicity were liver and spleen in mice, the nervous system in dogs and the liver in pigs.

Two year chronic studies were carried out in mice and rats and also a study over 2 1/2 years in rats using the mycelium. The no-effect levels ranged from 30-130 mg/kg diet. From the available studies an ADI of 0.05 mg/kg b.w. may be determined.

A one generation reproduction study in rats and embryotoxicity and teratogenicity studies in mice and rabbits revealed no adverse effects. Mutagenicity was absent when tested in a bacterial and two in vivo systems. Salinomycin had no effect on subsequent laying performance and hatchability of eggs when administered to young chicken for 16 weeks during rearing.

The use of salinomycin under the proposed conditions gives rise to small amounts of residues. The Committee considers however that there will be no hazard to the consumer if a withdrawal period of at least 5 days before slaughter is imposed.

2. Salinomycin is effective only against Gram-positive bacteria but no other microorganisms or helminths. No evidence for selection pressure or selection of enterococci with R-factors was found. No cross resistance to six other antibiotics used in human medicine was found. No resistance was induced in 10 different coccidia strains subjected to repeated passage. There appears to be no need for concern over the possible development of bacterial resistance.

3. Only about 1-5% of the microbiological activity in the feed appears in the broiler excreta because most metabolites are microbiologically inactive. The half-life of salinomycin in soil was 50 hours when measured microbiologically (limit of detection 0.01 mg/kg), only 1% remaining after 21 days. No data are available on leaching from soil or on the nature of the breakdown products in soil.

Salinomycin has a low toxicity for Daphnia (LC_{50} 24 hours: 4.3 mg/l) and fish (LD_{50} Idus idus 96 h: 30 mg/l). Salinomycin in the faeces of cattle reduced methane production by about 15% but in fresh pig manure it increased production by 5%. At a level of 8 mg/kg in soil nitrification was delayed but this level is about 1200 times the maximum possible concentration, if animal dung is used as fertiliser. Plant growth was slightly inhibited at doses of 12.5-200 mg/m^2 in a few crops only and no uptake in plants at 14 mg/kg soil was noted. These observations indicate that possible harm to the environment is unlikely.

4. In the light of the available evidence the Committee is of the opinion that the use of salinomycin in feedingstuffs for chickens, piglets, pigs and cattle, at the proposed levels, could be admitted provisionally provided a withdrawal period of at least 5 days before slaughter is imposed. A reassessment of this additive is envisaged when details of the metabolism become available.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF NARASIN IN FEEDINGSTUFFS FOR CHICKENS

Opinion expressed 14 April 1982

TERMS OF REFERENCE (November 1980)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of coccidiostat Narasin in feedingstuffs for chicken under the proposed conditions of use (see Background), result in the presence of residues in animal products? If so, what is the nature and the amount of these residues? Could these residues be harmful to the consumer?

2. Could the use of this additive affect the development of resistance in bacteria?

3. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

4. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

Narasin was the subject of a submission for inclusion in Annex II, Section B, of Council Directive 70/524/EEC of 23 November 1970, concerning additives in feedingstuffs (1), under the following proposed conditions for use :

(1) OJ No L 270 of 14.12.1970, p. 1

Species of animal : chicken for fattening.
Minimum and maximum content in complete feedingstuffs : 60-80 ppm (mg/kg).

OPINION OF THE COMMITTEE

1. Narasin is a polyether antibiotic produced by deep culture fermentation of a strain of Streptomyces aureofaciens. Its structure has been elucidated (Berg and Hamill 1978) and shown to be a monobasic carboxylic acid, containing five cyclic ether rings.

   In chicks given a single oral dose of ^{14}C Narasin, a mean recovery of radioactivity in the excreta of 99% (range 90-114%) was found within 2 days, of which 30% was unchanged ^{14}C Narasin. With the rat, 98.9% of total radioactivity was excreted in the faeces within 52 h following a single oral dose of ^{14}C Narasin (5% was unchanged Narasin), while the remainder was excreted in the urine. That absorption of the product from the digestive tract can take place is evidenced by the fact, that in rats with biliary cannulae 16% of an oral dose was excreted in bile within 24 h. Narasin is metabolised in both rat and chick to numerous metabolites. In rat faeces three of the most abundant comprise 4, 19 and 10% of total radioactivity: in chicken excreta equivalent values are 7, 4 and 3%. Six of the metabolites have been characterised by mass spectrometry, four being dihydroxynarasins and two trihydroxynarasins. Four or more of these metabolites are present in chicken liver. The rat produces the same metabolites as are found in chicken liver, and their presence has revealed no adverse effects during the subacute and chronic toxicity studies.

When Narasin was fed at 80 mg/kg complete feedingstuff, residues in chicken tissues following a one day withdrawal period ranged from 0.133 mg/kg (liver) to 0.084 mg/kg (skin) with none detectable in kidneys or lean tissue (limit of detection of the microbiological method : 0.005 mg/kg); after a three day withdrawal period there were no residues in kidney, lean tissue or fat, the amounts in liver and skin being respectively 0.044 mg/kg and 0.025 mg/kg. In further experiments total residues of Narasin plus metabolites, present in the edible tissue from a whole chicken, were, after a two day withdrawal period, estimated to be 0.006 mg (for 80 mg Narasin/kg complete feedingstuff) and 0.017 mg (for 100 mg Narasin/kg complete feedingstuff).

Acute toxicity studies show that for the chicken the LD_{50} value approximates to 50 mg/kg b.w., for mice and rats it lies in the range 15-20 mg/kg b.w., and for rabbits and dogs it is more than 10 mg/kg b.w. It is especially toxic to horses (LD_{50} 1 mg/kg b.w.). On the basis of a repeated dose study lasting 56 days, the no-effect level of Narasin for chickens was shown to be at least 80 mg Narasin/kg feed; at the higher levels tested, viz. 240 and 400 mg/kg, there were adverse effects on weight gain, feed comsumption and on various blood parameters. Two sub-chronic 90-day studies in mice showed no specific toxicity, with a no-effect level that appears to be higher than 10 mg/kg ration. The 90-days sub-chronic study in rats showed some haematological and biochemical changes which are difficult to interpret while the 2-year study revealed effects on body weight at all levels down to 7.5 mg/kg ration but no other significant findings. From the available studies the no-effect level for calculation of an ADI is 0.375 mg/kg b.w.

The use of Narasin under the proposed conditions does give rise to small amounts of residues. The Committee considers, however, that there will be no hazard to the consumer if a withdrawal period of at least 5 days before slaughter is imposed.

2. Narasin is inactive against gram-negative bacteria especially E. coli. In gram-positive bacteria (Streptococcus and Staphylococcus aureus) sensitivity to the antibiotic is occasionally very slightly decreased. None of the bacterial strains tested, even when they developed transient resistance to Narasin, showed resistance to a variety of clinical antibiotics tested. Hence there appears to be no need for concern over the possible development of bacterial resistance.

3. Narasin present in chicken litter does not degrade readily. After 18 months storage of chicken litter under field conditions, concentration of the product in the litter showed no change. However, in contrast, it appears to be readily degradable in soil. When Narasin was mixed with soil at 10 mg/kg, 90% degradation occurred within 22 days. Results of the same order have been obtained for poultry litter from birds fed narasin, which was incorporated in soil. Due to the rapid degradation of narasin in the soil nitrifying bacteria are not adversely affected.

Leaching of narasin from soil appears to be more dependant on soil pH than soil type, being significantly greater in basic soils (pH $\gtrsim$ 7.7). Since narasin is virtually insoluble in water, it would appear that soil leachate carrying it into surface waters would not present any hazard to aquatic life. Narasin has a low toxicity for Daphnia magna (no-effect level : 4 mg/l at 24 h exposure, 2.3 mg/l at 48 h; LC_{50} greater than 16 mg/l at 24 h, 8.0 mg/l at 48 h) and fish (LC_{50} at 96 h : 1.4-2.0 mg/l for Salmo iridens; 1.0-1.4 mg/l for Lepomis macrochirus). At a concentration of 1 mg/l, narasin inhibited

N-fixation in the blue green algae Anabaena flos aquae and partially so in the heterotroph bacterium Azobacter chroococcum, although the growth of neither organism was affected.

No narasin-related phytotoxicity occurred in the 14 species of plants tested in soils treated with chicken litter from birds fed narasin.

These observations indicate that possible harm to the environment is unlikely.

4. In the light of the available facts, the Committee is of the opinion that the use of Narasin in feedingstuffs for chickens, at the proposed levels, could be admitted provisionally provided that a withdrawal period of at least 5 days before slaughter is imposed. A reassessment of this additive is envisaged when full data on the metabolism in chickens become available.

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Dossiers Lilly Research Centre Ltd.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF NIFURSOL IN FEEDINGSTUFFS FOR TURKEYS

Opinion expressed 14 April 1982

TERMS OF REFERENCE (February 1978)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of nifursol as antihistomoniasis agent in feedingstuffs for turkeys, under the conditions of use authorized (see Background), result in the presence of residues in animal products? If so, what is the nature and the amount of these residues? Could these residues be harmful to the consumer?

2. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

3. In the light of the answers to the above questions, should the conditions of use authorized for this additive be maintained or should they be modified?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the twentieth Commission Directive of 7 December 1977 (2), Member States are authorized to use nifursol by way of derogation up to 31 December 1978, under the following conditions set out in Annex II, Section B, of the Directive:

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 18, 24.01.1978, p. 7

Species of animal : turkeys.
Minimum and maximum content in complete feedingstuffs : 75 ppm (mg/kg).
Other provisions : use prohibited at least 5 days before slaughter.

It was proposed to authorize this use at Community level at dose-levels of 50-75 mg/kg complete feedingstuff.

OPINION OF THE COMMITTEE

1. Studies of the metabolism of nifursol [3,5-dinitro-(5-nitrofurfurylidene)salicylic acid hydrazide] in turkeys and rats, using molecules labelled with ^{14}C either uniformly in both aromatic rings or only in the furan moiety, indicate that the compound is excreted mainly via the bile of the faeces. The main absorption site is the jejunum, some 30-50% of the absorbed material entering the enterohepatic circulation. In rats 80-85% appears in the faeces and 11% in the urine within 24 hours, whilst turkeys excreted 86% in the same period. Rats eliminate nifursol completely after 96 hours, with 2% appearing as labelled $^{14}CO_2$ derived from the furan moiety. Turkeys excreted 96-99% after 96 hours.

   Complex schemes for the metabolism in the turkey and rat have been proposed and some of the individual ultimate metabolites have been identified. In turkeys they arise by hydrolysis of the azomethine bond and by reduction of the nitrogroup in the furan ring followed by oxidative ring scission while maintaining the azomethine bond. In the rat only the furan ring is opened after reduction and hydrolysis of the hydrazine-furan bond. The turkey and the rat have only 2 of the proposed metabolites in common. Conjugation in turkeys occurs with pyruvic and glucuronic acid, in rats with acetic acid.

Residues in turkey tissues were determined by HPLC using an electron capture detector specific for nifursol but not detecting any metabolites (limit of detection 10 µg/kg). If ^{14}C-labelled nifursol was used at the proposed concentration of 75 mg/kg feed in turkeys, then residues in skin, liver and kidneys ranged from 0.35-0.60 mg/kg, with most of the nifursol being excreted in the bile. Residues in muscle were $<$ 0.1 mg/kg independent of the length of administration or withdrawal period. After 5 days withdrawal from feed residues in all tissues were $<$ 0.1 mg/kg using ^{14}C-labelled material. No detectable residues of unchanged nifursol were found in liver, kidney, muscle and skin of birds treated without a withdrawal period using the HPLC method (limit detection 10 µg/kg).

Orally administered nifursol is virtually non-toxic to rats, chicken and dogs in acute tests. Short-term studies in rats, dogs and turkeys showed the dog to be the most sensitive species, causing hepatotoxicity at high dose levels. A chronic 118 weeks feeding study in rats revealed no carcinogenic but some hepatotoxic effects. A three generation reproduction study in rats showed no adverse effects on reproductive function. From these long-term studies a no-effect level of 400 mg/kg diet could be established to serve for the evaluation of an ADI.

The fertility and hatchability of eggs was not affected by 4 months administration of 75 mg nifursol/kg feed. No teratology or embryotoxicity studies were performed. Studies on mutagenicity in several Salmonella typhimurium strains were negative.

It would appear that the proposed use, which includes a 5 day withdrawal period, presents no risk to the consumer.

2. Nifursol has only weak antibacterial activity. The mean inhibitory concentrations for 12 species of soil microflora demonstrated that significant antibacterial activity was present only against B. subtilis. The level of nifursol in turkey excreta is well below the effective level against the most sensitive soil bacteria tested. The rapid degradation of nifursol in turkey excreta (20 mg/kg decomposing in 10 days) with a half-life of 8.4 days, together with the dilution when excreta are spread on soil, make any selective effects on soil microflora unlikely.

Studies with aquatic organisms, relevant to the assessment of environmental effects, showed that nifursol is only moderately toxic to Daphnia magna ($LC_{50} > 10$ mg/l) and Poecilia reticulata (LC_{50} 24-96 hours : 6-10 mg/l). Algae were not investigated specifically. No adverse effects on trout were noted. Nifursol is unstable when aqueous solutions are exposed to UV light, 82% being decomposed in 8 hours. Nifursol is rapidly decomposed in soil, its concentration falling to 50% in 3 days and complete decomposition occurring within 77 days. No more than 9% of nifursol in soil can be removed by leaching. The nature of the decomposition products in soil is not known.

The instablility of the compound in excreta, soil and aqueous solutions exposed to UV light makes it unlikely to become a hazard for the environment. The low antibacterial activity suggests no adverse effects from nifursol on soil microorganisms and makes it unnecessary to determine its effects on nitrifying and methanogenic bacteria.

3. In the light of the available information the Committee is of the opinion that the use of nifursol in feedingstuffs for turkeys, at use level of 50-75 mg/kg (ppm) should be maintained subject to a withdrawal period of not less than five days before slaughter.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF NICARBAZIN IN FEEDINGSTUFFS FOR POULTRY

Opinion expressed 14 April 1982

TERMS OF REFERENCE (December 1977)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of the coccidiostat nicarbazin in feedingstuffs for poultry under the conditions of use authorized (see Background), result in the presence of residues in animal products? If so, what is the nature and the amount of these residues? Could these residues be harmful to the consumer?

2. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

3. In the light of the answers to the above questions, should the conditions of use authorized for this additive be maintained or should they be modified?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the twentieth Commission Directive of 7 December 1977 (2), Member

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 18, 24.01.1978, p. 7

States are authorized to use nicarbazin, by way of derogation up to 31 December 1978, under the following conditions set out in Annex II, Section B, of the Directive :

Species of animal : poultry
Minimum and maximum content in complete feedingstuffs : 100-125 ppm (mg/kg).
Other provisions : use prohibited from laying age onwards and at least seven days before slaughter.

OPINION OF THE COMMITTEE

1. Nicarbazin is an equimolecular crystalline complex composed of 67.4-73.0% 4,4'dinitrocarbanilide (DNC) and 27.7-30.0% 2-hydroxy-4,6-dimethylpyrimidine (HDP).

   Studies of the metabolism of nicarbazin were carried out using ^{14}C-labelled molecules either in the DNC moiety or in the HDP moiety in colostomised chickens, permitting separete collection of urine and faeces. With chickens fed during 3 days on a diet containing 125 mg of the product/kg feed 96-110% of the radioactivity was excreted within the 3 following days in faeces and urine; 9,7% of the DNC and 98,7% of the HDP were recovered in the urine. The HDP component is absorbed and excreted or metabolised more rapidly than the DNC component. Immediately on withdrawal, residues in the various tissues and organs ranged from 1.6 to 3.2 mg/kg for HDP and from 3.3 to 32.4 mg/kg for DNC. Five days after withdrawal, the residues has disappeared from all tissues and organs (limit of detection : 0.02-0.10 mg/kg) except the liver where traces of DNC were still present on the 8th day.

In other experiments on chickens feed on diets containing 100-800 mg nicarbazin/kg feed, HDP could only be detected in the liver and had disappeared 24 hours after withdrawal of the supplemented feed, except at the dose-level of 800 mg/kg feed. DNC residues in the various tissues and organs had disappeared 48 hours after withdrawal except in the liver where they ranged from 2.4 to 4.7 mg/kg according to the dose-level in the feed (100-800 mg/kg feed). Small amounts of the metabolite diacetylamidocarbanilide, derived from DNC, were also detected in the liver.

After prolonged feeding of nicarbazin to laying hens (50-400 mg nicarbazin/kg feedingstuff during 32-36 weeks) residues of DNC of 10-32 µg/ml were found in egg yolks.

There are few data available on residues resulting from the use of nicarbazin under presently authorized conditions. They refer to DNC (the longer persisting component of the complex) in chicken tissues. These residues were estimated five days after withdrawal at 0.1-0.2 mg/kg in the liver and less in other tissues. It should be noted, however, that these values were obtained by extrapolation of results obtained by a polarographic method of low sensitivity (limit of detection : 1 mg/kg).

Nicarbazin was investigated in short- and long-term toxicological studies in laboratory animals. In the long-term and reproduction studies a mixture of DNC and HDP (ratio : 3/1) was used. These studies were carried out in the period 1965-1970 and do not satisfy all the requirements of current toxicological testing; nevertheless, they suffice for the evaluation of the ADI. The no-effect level was estimated at 50 and 60 mg DNC/kg body weight in the rat and the dog

respectively. Using a safety factor of 250 (to compensate for some experimental gaps), the ADI was evaluated at 0.20-0.24 mg/kg body weight. There is thus a large margin of safety between the ADI and the amount of residues which may persist in edible chicken products after a withdrawal period of at least 7 days ($<$ 0.2 mg DNC/kg animal product).

2. No data were available to the Committee on the biodegradation of HDP, DNC and their possible metabolites in poultry manure, soil and water nor on their toxicity to soil micro-organisms. As DNC exhibits only slight solubility in water (0.02 mg/l), a contamination of water streams seems unlikely. The toxicity of DNC and HDP to water organisms was tested in the unicellular alga Chlorella pyrenoïdosa, the water flea Daphnia magna and in two fish species : Poecilia reticulata and Salmo gairdnerii. The acute toxicity of HDP was in all cases very low. For DNC, no toxic effects were observed at the concentration of 0.02 mg/l (maximum solubility in water).

3. In the light of the available information, the Committee is of the opinion that the use of nicarbazin as feed additive should be limited to feedingstuffs for fattening chickens. No data are available to justify the use of this product in chickens reared for laying or other poultry species. The dose-levels of 100-125 mg/kg complete feedingstuff and the withdrawal period of at least 7 days before slaughter appear acceptable. However, a reassessment of this additive is needed, after more extensive studies on the metabolism of nicarbazin in chickens, biodegradation of excreted products and their possible effects on soil micro-organisms have been carried out.

Note

According to Neshavarz and McDougald (1981), chickens fed a diet containing nicarbazin are more sensitive to stress caused by heat. Under these conditions, there is an increase of mortality in chickens when 18-29 days old.

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Dossiers Merck and Co. Inc.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF COPPER COMPOUNDS IN FEEDINGSTUFFS

Opinion expressed 15 April 1982

TERMS OF REFERENCE (December 1977)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the addition of copper compounds to feedingstuffs result in significant effects on growth and/or on the conversion rate of the feed? In this respect, are the maximum contents of copper authorized (see Background) justified?

2. What is the relationship between the copper content of the animal ration and the residual amount in animal tissue and organs? Are the residues resulting from the authorized conditions of use free from risks to the consumer?

3. What is the relationship between the copper content of the ration of the animal and the amount excreted? Can the amount of copper excreted, resulting from the authorized conditions of use, be prejudicial to the environment, and if so what is the nature of the risks?

4. In the light of the answers to the above questions, should the conditions of use authorized for the addition of copper compounds to feedingstuffs be maintained or should they be modified?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by the twentieth Commission Directive of 7 December 1977 (2), the addition of copper compounds is authorized at Community level under the following conditions set out in Annex I, Section (i), of the Directive :

Additive	Species of animal	Maximum content (ppm (mg/kg) feedingstuff)
Copper acetate Basic copper carbonate monohydrate, Copper chloride Copper oxide Copper sulphate	Swine Other species	125 (total Cu) 50 (total Cu)

Furthermore, Member States are authorized to use, by way of derogation up to 31 December 1978, complete feedingstuffs for swine with a maximum copper content (total Cu) of 200 mg/kg (Annex II, Section D (a) of the Directive).

OPINION OF THE COMMITTEE

1. Copper is a trace element essential for physiolocigal equilibrum. The physiological copper requirements of animals vary according to the species; generally speaking, they seem to be adequately covered by the copper content of vegetable and animal origin which make up their

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 18, 24.01.1978, p. 7

daily rations (Maletto 1959, Jacquot et al. 1964, Boccard 1980)*.

1.1 The addition of copper compounds to feedingstuffs in quantities exceeding physiological requirements has favourable effects, similar to those of growth promoters, on weight gain and feed conversion in certain animal species. The effects have only been thouroughly studied in pigs. The results of numerous feeding trials involving large numbers of pigs, designed to investigate the effects of copper in relation to feed composition, feeding systems and other variables, have been published and discussed in comprehensive reviews of the literature (Braude 1967 and 1975, Meyer and Kröger 1973, CLO 1978, UKASTA 1978, Brajon et al. 1980, Proc. EEC Workshop 1981).

1.1.1 According to data reported by Meyer and Kröger (1973), the addition of 125 and 250 mg of copper (in sulphate form) per kg feed for early-weaned piglets between 3 and 12 kg liveweight produces average increases in weight gain of 22.2% and 28% respectively, and average improvements of 14.3% and 15% respectively in the feed conversion ratio. These values fall to 10.2% and 14.2% (weight gain) and 2.6% and 3.9% (feed conversion) in piglets between 5 and 25 kg liveweight.

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* The copper contents of daily rations given below, in mg/kg dry matter, are regarded as sufficient for physiological requirements: piglets: 6-10; adult pigs: 6; calves: 9; adult cattle: 10-14; sheep: 5; chickens (up to 8 weeks): 2; adult chickens: 3,2; turkeys: 4; rabbits: 3-5 (Jacquot et al. 1964, Neathery and Miller 1977, Brajon et al. 1980, Boccard 1980).

But the investigators stress that no conclusion may be drawn as regards the optimum copper content of the feed because of the range of variation of the individual values. Aumaître (1981) and Braude (1981) find that, up to a weight of 20 kg the effects of the addition of 250 mg copper per kg of feed are variable and sometimes nil.

1.1.2 The addition of 100-150 mg of copper (in sulphate form) per kg of feed for fattening pigs produces, according to data reported by Meyer and Kröger (1973), an average increase of 4.7% in weight gain and an average improvement of 2.3% in feed conversion in the period between 15-30 kg and 90-110 kg liveweight. The addition of 250 mg copper per kg feed procures an average increase of 7.4% in weight gain and an average improvement of 4.6% on the feed conversion ratio. The corresponding values for 250 mg copper per kg feed reported by Braude (1967 and 1975) are as follows: 8.1 and 5.4% (trials between 1955 and 1965); 9.1 and 7.4% (trials between 1965 and 1975). According to trials carried out in the Netherlands (CLO 1978), the dose/effect ratio is linear at least up to 270 mg of copper/kg feed for the weight range between 20 and 110 kg liveweight. The results of a statistical analysis of data in 129 publications (period between 1953 and 1975) carried out by UKASTA (1979) indicate that the optimum copper level is 224 mg/kg feed and that this achieves an average improvement of 6.5% on weight gain for the growth/fattening period.

1.1.3 The range of variation underlying these average values is attributable to a number of factors; in particular, the type and quantity of protein in the ration, and the quantities of iron, zinc, molybdenum and sulphur in the feed play a significant role. The effects of these factors are more variable when the

copper content of the ration is low; variability tends to become insignificant at the level of 250 mg copper/kg feed (Maletto et al. 1980).

According to the data reported by Meyer and Kröger (1973), the improved growth performance observed in piglets (see Section 1.1.1 above) was not obtained when the feed contained a high proportion of skimmed milk powder. The improvements in weight gain recorded in fattening pigs at the end of the first rearing period (up to 60 kg liveweight) were distinctly better in pigs receiving feed containing fish or meat meal than in those receiving soya cakes, irrespective of the quantity of copper added (100-150 mg/kg and 250 mg/kg). On the other hand, the phenomenon was observed in reverse in the final fattening stage.

1.1.4 In cattle, the addition of copper salts exceeding physiological requirements to feed seems to have little effect; in sheep it is harmful. (See Section 3.2.2 below.)

1.1.5 In poultry and rabbits the addition of copper to feed has produced variable improvements in growth, depending on the composition of the ration (Beede and Sullivan 1975; Omole 1977 and 1980). A drop in mortality due to dissecting aneurysm has been observed in turkeys after the administration of a high protein ration supplemented with 120 mg of copper/kg feed (Hill 1969, Guenther and Carlson 1975).

1.2 According to these data, the maximum copper contents authorized in the Community in animal feed appear to be justified, economically, only in pig management. It has been established that there is no significant difference between the effectiveness of copper sulphate, carbonate, chloride or oxide (Braude 1965).

For sheep, the authorized maximum copper content is unacceptable for toxicological reasons; it should be reduced to 15-20 mg/kg complete feed. For other species, the maximum content of 50 mg/kg complete feed seems acceptable but it is not substantiated by sufficient data.

2. Copper levels in the animal organism vary according to species and according to the tissues or organ concerned. The highest levels are found in the liver. Copper ingested with feed is absorbed only partially. It circulates in the blood in the form of complexes bound to albumins and amino acids (Ludvigsen 1981). It is stored, as cuproprotein, in the liver and, to a small extent, in the kidneys, the spleen, the lungs and the muscles. The liver regulates the homoeostasis of the copper firstly by synthesis of the coeruloplasmin in the blood and by excreting excess copper as complexes through the bile. Only a small proportion is eliminated via the urine. The rate of copper retention in pigs is very low and varies between 2-10% (Bowland et al. 1961).

2.1 The data available on the ratio between the copper content of the feed and the quantity of residual copper in the organs and animal tissues relate only to pigs.

2.1.1 In fattening pigs receiving a diet supplemented with copper, the copper level in the liver rises with the copper content in the feed and the duration of the copper-supplemented diet. According to data collected by Meyer and Kröger (1973), this process of accumulation is not linear (see table below).

Relation between dietary copper and liver copper in pigs (90-110 kg) (Meyer and Kröger, 1973)

Quantity of Cu added to the diet (mg/kg)	Cu concentration in the liver (mg/kg of wet tissue)	
	Average	Range of values
0	12	1,6 - 57
60	17	12,6 - 29
125	57	7,3 - 273
250	256	8,0 - 890
500	817	- 1556

The ratio between the average quantity of copper accumulated in the liver and the quantity ingested increases appreciably when the ingested quantity exceeds 125 mg of copper/kg feed. The copper level in the liver drops when copper supplementation is stopped (Meyer and Kröger 1973, Castell et al. 1975, Lillie et al. 1977, Braude 1978). In pigs receiving copper (in the form of sulphate) at a concentration of 250 mg/kg feed, the copper level in the liver falls to less than half when supplementation is discontinued either 14 days before slaughter or as soon as the middle of the fattening period is reached (Braude 1978).

The average values quoted by Meyer and Kröger (see table) are distinctly higher than those collected by other authors. According to Braude and Ryder (1973) the average copper level in the liver is 286 mg/kg dry matter (=95 mg/kg wet tissue) in pigs receiving a feed ration supplemented with 250 mg Cu/kg. According to Nadazin et al. (1977), in pigs receiving 50 and 250 mg of copper/kg feed, the copper content in the liver was 8.9 and 88.1 mg/kg of wet tissue. According to trials carried out in the Netherlands (CLO 1978) the copper level in the liver is 150 mg/kg dry matter (=50 mg/kg wet tissue) in the case of a feed ration supplemented with 200 mg/kg.

2.1.2 The wide range of variation of the values is explained to a large extent by the variability of the composition of feed rations on different livestock units. The type and quantity of proteins in the feed ration and the quantity of trace elements (zinc, iron and molybdenum) and sulphur seem to be decisive. It has been established that copper retention by the liver is two to four times higher in the case of animal-protein-based rations than in the case of vegetable-protein-based rations (Combs et al. 1966, Parris and McDonald 1969, Drouliscos et al. 1970). On the other hand, copper retention is inhibited by the addition of zinc and iron to the feed ration (De Goye et al. 1971). The low levels of copper in the liver recorded in the Dutch trials (CLO 1978) were attributed to appropriate supplementation with zinc and iron and the lack of animal proteins in feed rations. This type of feed for pigs is now commonly used.

The chemical and/or physical form in which copper is ingested can also influence the degree of retention (Bekaert et al. 1967, Kirchgessner and Grassmann 1970).

2.2 Copper requirements and copper metabolism and toxicity in man are well known (Mason 1979, Bories 1981). According to a WHO report (1971), daily intake of 0.5 mg Cu/kg body weight may be regarded as a maximum acceptable dose provided that the diet contains appropriate quantities of zinc, iron and molybdenum. According to various surveys in Western countries, the average copper content of the human diet rarely exceeds 1-2 mg/kg food. There is therefore a large margin of safety between the quantity of copper ingested each day and the maximum acceptable daily intake (30 mg for a subject weighing 60 kg). Occasional consumption of pig's liver possibly containing a large quantity of copper

resulting from the authorized maximum level of copper in pig feed (200 mg total Cu/kg complete feed) is therefore not likely to be hazardous for the consumer. However, it seems inadvisable to use liver with a high copper content for feeding young children in view of the fact that large quantities of liver are often incorporated in dietary preparations for daily consumption (Bories 1981).

Changes in the quality of pork fat (particularly a drop in melting point and an increase in the unsaturated fatty acid content) attributable to an increase in the activity of the hepatic enzyme stearyl CoA desaturase induced by excess copper, have been observed only in certain circumstances and where feed rations have contained more than 200 mg Cu/kg (Brajon et al. 1980).

3. When copper supplements are fed continuously over a long period, there is a direct relationship between the amount of copper ingested and the amount excreted (Brajon et al. 1980, CLO 1978).

3.1 According to calculation by Feenstra et al. 1979, if pigs are administered copper supplements in their feed at the rate of 200 mg/kg, about 68 g of copper/animal will have been excreted by the time market weight is reached. A total of 144 g copper per fattening place will thus be excreted each year. According to a report published by the E.C. Commission (1978), if weaned piglets are fed on a diet supplemented with 200 mg Cu/kg from the first to the seventh week and then 125 mg Cu/kg until the nineteenth week, then 86 g will be excreted per fattening place per year.

3.2 Copper administered to pigs reappears in the environment as a result of the use of slurry and manure as fertilizer and of waste

water purification plants. It accumulates mainly in the surface layers of soil (Jones et al. 1967, Dalgarno and Mills 1975, Reith et al. 1979, Univin 1980, McGrath et al. 1980).

3.2.1 Although knowledge as to its different forms in pig faeces and slurry is incomplete (Braude 1980), copper is supposed to be present partly as sulphide which, under aerobic conditions in the soil, could be partially converted into copper sulphate. It could then be absorbed in ionized form by plants (CLO 1978). Given the complex nature of the subject and the fact that the data at present available are insufficient and often contradictory (Kiekens and Cottenie 1981), it is difficult to know in what forms copper is bound by the different types of soil and what factors govern the bioavailability of copper for plants and, subsequently animals.

In the soil, the bioavailability of trace elements in general and of copper in particular depends on their interaction with the various soil components such as organic matter, colloidal mineral particles (clay) and amorphous Fe, Al and Mn carbonates. The interaction features several parameters, in particular the concentration of ions in solution in the soil, the type and frequency of adsorption sites (solid phase), the concentration of ligands capable of forming organomineral complexes (fulvic and humic acids), the pH and the redox potential, etc.

The extraction solutions which are used to simulate the absorption of copper by plants vary, making comparison difficult. Using 0.43 N nitric acid, between 60% and 80% of the copper from swine excreta would appear to be available in the soil to plants (Batey et al. 1972). With 0.05 M NH_4-EDTA,

30-45% would appear to be bioavailable (Unwin 1981). With aqueous 0.5 M EDTA, 100% would be extractible (McGrath 1981).

The amount of copper taken up by plants depends on their genotype, the nature and physico-chemical properties of the soil, on micro-organism activity and the type of compound concerned (Meyer and Kröger 1973, CLO 1978, Feenstra et al. 1979, El Bassam 1979, Davis 1981). According to Feenstra et al. (1979), the amount removed annually by crops varies between 15 g and 80 g Cu/ha. The amount removed by an average harvest on sandy oil can be put at 50 g Cu/ha.

Little is known about soil leaching. According to data reported by Meyer and Kröger (1973) it would account for something in the order of 30 g Cu/ha. In Denmark, it is estimated that in practice 8-19 g/ha are removed by leaching (Kofoed 1981). Köhnlein (1972) used lysimetric readings to analyse the leaching of copper resulting from a rainfall of 270 mm in a heathland podzol and found a lixiviation of 85 g Cu/ha (topsoil 30 cm). In two brown earths with different basic saturation he found lixivations of 86-270 g Cu/ha, soils with a low basic saturation having a higher lixivation. On grassland, only the loss through leaching must be taken into account since the copper present in crops is recycled through excreta of grazing animals. The estimated overall removal through cropping and leaching would thus be 30 g Cu/ha on grassland and 80 g/ha on arable land (Feenstra et al. 1979).

Leaching of copper by rain and run-off could, in the long run, lead to undesirable concentrations of copper in ground water and bodies of surface running water, but too few data are at present

available to permit a definitive conclusion concerning the possible environmental hazards of the leaching of copper.

3.2.2 Copper is an element essential to plants. Plants sometimes show signs of deficiency if there is a shortage of copper in the soil (Jacquot et al. 1964, Boccard 1980, Brajon et al. 1980, Unwin 1981). In soils with a high copper content, plant association may be disturbed, with an increase in the frequency of the more resistant species. Excess copper may be toxic (Scurti 1957).

Field data on the phytotoxicity of copper in the soil are scarce, and caution is required when interpreting the results of pot trials, especially where copper salts are added directly (Unwin 1981, Davis and Beckett 1980). The phytotoxicity of copper depends on the type of crop, the physico-chemical properties of the soil and its pH, the quantity of soluble copper in the soil and the possible interaction between copper and other minerals, such as Mo, SO_4, Zn, Cd, Fe (Williams 1975, Kofoed 1981). The fact that there are so many variables and that so many different extraction solutions are used for determining phytotoxicity thresholds accounts for the inconsistency of the results published (Adas 1971, Commission of the EC 1978, CLO 1978, Feenstra et al. 1979, Coppenet 1980). It is preferable to use plant tissue as a reference. The copper content in the leaves is a sensitive indicator of phytotoxicity in most plants.

The phytotoxicity threshold is generally of the order of 20 mg Cu/kg foliar dry matter (Davis and Beckett 1980). Above that level there is a risk of falling yields for certain species (Finck 1976, Beckett and Davis 1977 and 1978, Kofoed 1981).

However, some plants or parts of plants are capable of tolerating high quantities of copper (20-30 mg/kg dry matter). Examples are potato tops, clover and swede tops (Reith et al. 1979), carrots and buckwheat (El Bassam 1979), chenopodiaceous plants (sugarbeet roots and leaves) and ceral leaves (Davis 1981). There is a gradual rise in the copper content of plants as the accumulation of copper in the form of slurry increases (Bachtaler et al. 1974, McGrath et al. 1980, R.J. Unwin 1981). The rise in plant copper content varies considerably according to species and may even be insignificant (Martens et al. 1979, Poole 1981).

3.2.3 As regards the toxicity of copper for the higher animals, resulting from the spreading of pig slurry containing copper on grazing land, there is a risk only in the case of sheep. Small quantities of copper (12-20 mg/kg dry matter) in the forage material of sheep (Texel lambs are particularly sensitive) can induce signs of intoxication, in particular hemolytic disease (Hill 1977, Vink 1978, Hadenfeldt 1978). But the presence of molybdenum, iron or zinc in the feed counteracts the toxic effects of copper (Ferguson 1943, Dick and Bull 1945, CLO 1978, Lamand 1981). Ingestion of copper as a result of eating soil should not be overlooked, especially in the case of sheep (Field and Purves 1964, Healy 1967, Suttle et al. 1975, Poole 1981). According to Feenstra et al. (1979) the presence of 15-20 mg of 0.43N nitric-acid-soluble copper/kg of soil already constitutes a risk for grazing ovine animals.

3.2.4 Available data on the effects of copper on soil fauna and flora are very incomplete. With regard to earthworms, the data are

conflicting. According to Van Rhee (1975), earthworm populations in sandy soils are depleted after 10 years of application of pig slurry with a high copper content. The minimum copper level affecting reproduction is 80 mg/kg soil (soluble in 0.43N nitric acid). According to Unwin (1981), the earthworm population in plots treated with pig slurry containing 212 kg Cu/ha doubled in the space of four years. El Bassam (1979) observed that an increase in the soluble copper content brought a gradual decrease in the activity of micro-organisms in the soil, thus jeopardizing the complete breakdown of organic matter. The action of copper on nitrogen-fixing bacteria and on methane formation in manure and slurry is not known.

3.2.5 Studies of the selection of bacteria resistant to certain antibiotics have shown that the feeding of pigs with a diet containing quantities of copper exceeding essential requirements encourages the selection of strains of E. Coli resistant to chloramphenicol (Gedek 1981).

3.2.6 The toxicity of copper for aquatic organisms depends on many factors, in particular temperature, pH and water hardness and the quantity of dissolved salts and oxygen (Brajon et al. 1980). For algae and plankton (Gammarus pulex, Tibiflex rivolorum, Heptagenia lateralis, Chironomus thummi), the lethal concentrations of copper range from 0.25 to 0.5 mg/l (Liepolt and Weber 1958, FAO 1968). For freshwater fish, the lethal concentrations range from 0.02 to 1.0 mg/l (Erichsen Jones 1964, FAO 1968). An increase in the copper content of water has an inhibiting effect on aquatic micro-organisms and thus slows down tne breakdown of organic substances (Maletto 1981).

Measures to protect surface water and fresh water against pollution have been taken at Community level under Council Directive 75/440/EEC of 16 June 1975 concerning the quality required for surface water intended for the abstraction of drinking water in the Member States (1) and Council Directive 78/659/EEC of 18 June 1978 on the quality of fresh waters needing protection or improvement in order to support fish life (2). Under these directives, the copper contents of surface waters must lie between 0.02 and 1 mg/l, while those of fresh water must not exceed 0.04 mg/l.

3.2.7 In intensive farming, the copper present in the feeds and excreted in the faeces may show an unfavourable interference in the functioning of the waste water purification plant. This negative effect would begin to appear at a concentration of 10 mg/l and would be magnified with the increasing concentration of copper in the sludge (Assozeni, unpublished research).

3.3 Although, under the present authorized conditions of use, the quantities of copper excreted and spread on arable land and grazing land do not seem to have had any prejudicial effect on grazing animals (except sheep), plants or fauna and flora in the soil and water there is a potential risk that the copper toxicity thresholds for susceptible animal and plant species will sooner or later be attained as a result of copper build-up in the soil. In this connection, animal excreta are not the only determining factor. Other sources of copper such as sewage sludge, household refuse compost and certain pesticides may make a significant contribution.

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(1) OJ No L 194, 25.7.1975, p. 26
(2) OJ No L 222, 14.8.1978, p. 1

4. In the light of the foregoing, the conditions under which the copper may be used in animal feed in the Community are economically justified in pig production and acceptable for other animal species except sheep.

Under these conditions of use, the use of copper has no hazardous effect on consumer health, although it is not recommended that liver with a high copper content be used as an everyday food for young children.

However, the application of animal excreta and slurry containing high quantities of copper cannot be considered as free from long-term risks for the environment. Excess levels of copper in the soil will inevitably have deleterious effects on the flora and fauna and on the yield of certain crops.

On the grounds of prudence, the Committee is of the opinion that the addition of copper compounds to animal feed should be limited to ensure that maximum levels of <u>total copper</u> do not exceed the following values :

125 mg/kg in complete feed for piglets and pigs;
20 mg/kg in complete feed for sheep;
50 mg/kg in complete feed other animal species.

Furthermore, the Committee recommends that no copper should be added to feedingstuffs for sheep except in cases of nutritional deficiency. The maximum level of 20 mg Cu/kg complete feed should never be exceeded; in certain circumstances and for some strains this level may already be harmful.

The proposed values should be reviewed at a later date in the light of additional data on the effectiveness of copper for animal species other than pigs and the effects of copper build-up and the leaching that may ensue.

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SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF CARBADOX IN FEEDINGSTUFFS FOR PIGS

Opinion expressed 7 July 1982

TERMS OF REFERENCE (March 1981)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the toxicity and the possible effects on health of the exposure to carbadox. The Commission considers it desirable that the following aspects especially be evaluated :

(a) carcinogenic potential of carbadox and its metabolites (dose/effect relationship, classification of the product according to the nature of the effects, etc.);

(b) risks inherent in exposure to carbadox dust under practical stock-farming conditions and opinion on the protective measures.

BACKGROUND

In its opinion expressed on 6 July 1978 (*), the Scientific Committee considered that carbadox can be used without risks for the health under determined conditions. Some arguments have been developed recently against this opinion, so that a further consultation of the Scientific Committee became necessary.

(*) Commission of the European Commuties. Reports of the Scientific Committee for Animal Nutrition, Second Series, EUR 6918 (1980), p. 7.

OPINION OF THE COMMITTEE

(a) Carbadox is structurally related to the know carcinogen 4-nitroquinoline-1-oxide. It is hepatotoxic causing nodular hepatic hyperplasia in a dose-related manner in chronic studies in rats down to a dose level of 2.5 mg/kg b.w./day. At the lowest dose level tested (1 mg/kg b.w./day) a lower incidence of hepatic nodular hyperplasia was seen when compared to controls. However, the number of animals in each group was too small to establish 1 mg Carbadox/kg b.w./day with certainty as the true no-adverse-effect level. The findings in two long-term feeding studies on rats point to carbadox being a hepatocarcinogen of low potency. These studies were, however, of inadequate design in the light of present-day guidelines. Hepatocellular carcinomas appeared only at the highest level tested (25 mg/kg b.w.). The compound has also been shown to be mutagenic in *in vitro* and *in vivo* tests. No evidence for toxicity or carcinogenicity was seen in a one-year study in guppies (*Poecelia reticulata*). Relay toxicity tests on rats extending over 3 generations and on dogs over 87 months showed no adverse effects.

The short-lived metabolite desoxycarbadox was a potent carcinogen in a fifteen months feeding study in rats with some evidence of a dose-response relationship. The metabolite quinoxaline-2-carboxylic acid, the compound persisting as residue in the liver of treated pigs, and detectable in the urine exposed indiduals, has been shown to be non-carcinogenic in an adequate 29 months study. The highest dose level studied was 100 mg/kg b.w. The other metabolite,

methylcarbazate, which appears temporarily in the urine of treated pigs, has also been found to be non-carcinogenic in a 2-year feeding study in rats. This study was, however, carried out on groups of only 24 males and females per dose level. Moreover, survival was comparatively poor. The highest dose level of methylcarbazate investigated was 10 mg/kg b.w./day, corresponding to a dose of $>$ 25 mg carbadox/kg b.w./day.

(b) It has been established that, following administration of carbadox, residues in food of animal origin are of low toxicity for the consumer once the original carbadox and its metabolite desoxycarbadox have been metabolised, i.e. 72 hours after administration of the product. The withdrawal period of at least 4 weeks before slaughter, imposed by the EEC regulation, therefore ensures the safety of the consumer, particularly in the light of the results of the relay toxicity tests.

With regard to the exposure to carbadox dust in handling premixes and feedingstuffs, the Committee expressed the view in its previous evaluation (Commission of the European Communities, EUR 6918) that carbadox was available commercially as a premix with a satisfactory physico-chemical specification. According to this specification the inert ingredient soya oil prevents the formation of dust during preparation of the premix and the feedingstuff. These products could therefore be handled safely and with negligible risks to agricultural workers, particularly if the feedingstuff was in pellet form.

Additional studies on agricultural workers and on pigs were undertaken at the request of the Committee with feedingstuffs containing

50 mg/kg feed of carbadox prepared from a 10% premix and used in meal form to obtain a maximum dust level in the ambient air during the preparation and distribution of pig feed. Dust inhalation was studied in animals and farm workers exposed for 15 days using test filters. The urine of both pigs and exposed farm workers was examined for the presence of the carbadox metabolite quinoxaline-2-carboxylic acid as evidence of systemic absorption.

The average inhalation exposure of farm workers was found to be 0.05 mg carbadox/kg bodyweight in 24 hours as measured by deposition on test filters. No quinoxaline-2-carboxylic acid was found in the urine of either exposed farm workers preparing and handling the feed or of treated pigs (limit of detection 10 ug/l).

These findings confirm the opinion of the Committee stated earlier, that the risk to health incurred by farm workers is negligible under practical conditions of preparation and distribution of animal feed containing carbadox, provided that defined specifications are being fulfilled. This opinion refers however to carbadox premixes, the documentation of which was available to the Committee, i.e. commercial premixes containing up to 10% active substance and specially formulated with ingredients preventing the formation of dust and fulfilling defined specifications. The evaluation of preparations containing carbadox and differing in specification would require that these products be tested in a manner similar to that described for the product used in the above investigations.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF LERBEK (*) IN FEEDINGSTUFFS FOR POULTRY

Opinion expressed 17 November 1982

TERMS OF REFERENCE (July 1978 expanded in October 1981)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

A. Chickens

1. Does the use of the coccidiostat Lerbek (*) (premix containing 100 parts of meticlorpindol and 8.35 parts of methylbenzoquate) in feedingstuffs for chickens, under the conditions of use authorized (see Background), result in the presence of residues in animal products? If so, what is the nature and the amount of these residues? Could these residues be harmful to the consumer?

2. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

3. In the light of the answers to the above questions, should the conditions of use authorized for this additive be maintained or should they be modified?

B. Turkeys

1. Does the use of Lerbek (*) in feedingstuffs for turkeys, under the proposed conditions (see Background), result in residues in animal

(*) registered trade name

products or excreted products which are different from those resulting from its use in chickens?

2. If so, could these residues or excretion products be prejudicial to the consumer or the environment?

3. In the light of the answers to the above questions, are the conditions proposed acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the thirty-eighth Commission Directive of 16 July 1981 (2), Member States are authorized by way of derogation to use Lerbek (*) under the following conditions set out in Annex II, Section B, of the Directive :

Species of animal : chickens for fattening.
Minimum and maximum content in complete feedingstuffs : 110 ppm (mg/kg).
Other provisions : use prohibited at least five days before slaughter.

An extension of the use of Lerbek (*) under the following conditions has been proposed :

Species of animal : turkeys for fattening.
Maximum age : 12 weeks.
Minimum and maximum content in complete feedingstuffs : 110 ppm (mg/kg).
Other provisions : use prohibited at least five days before slaughter.

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(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 231, 15.08.1981, p. 30
(*) registered trade name

OPINION OF THE COMMITTEE

A. Chickens

1. The metabolism of meticlorpindol (MCP) has been studied in rats, dogs and rabbits after oral administration. The product is partially absorbed. In rats and dogs, about 38 to 56% of the administered dose was excreted in the urine and 42 to 55% in the faeces. Unchanged MCP was one of the three major compounds identified among the products excreted in the urine. With rabbits, some 98% of the dose was excreted in the urine; almost half was as MCP and the remaining part as a mixture of hydroxylated MCP and its O-glucuronide derivative. Studies using ^{36}Cl-labelled MCP showed that there is virtually no breakdown of MCP in the tissues.

The metabolism of methylbenzoquate (MBQ) has been studied in rats and chickens, using ^{14}C-labelled molecules. In rats, 95% of the administered dose was excreted in the faeces and 0,2 to 1,0% in the urine within 4 days. Within 3 days about 1% of the dose was excreted in the bile, a large part of it in the form of an identified metabolite. In metabolism studies with surgically modified chickens, 80-85% of the radioactivity was excreted in the faeces (the greates part being as MBQ), and 1% in the urine. Several metabolites excreted have been identified.

Various studies on tissue residues have been carried out in rats, chickens and rabbits. In chickens fed Lerbek (at the authorized level of 110 mg active ingredient/kg feedingstuff) for 10 days, tissue levels of MCP after 5 days, but not at 3 days were undetectable (limit of detection : 0.05 mg/kg). Residues of MBQ

were not detected in any sample. The elimination of MCP residues in muscle and liver was confirmed in studies with chickens fed 100 mg MCP/kg feedingstuff for six days. In chickens fed MBQ at 20 mg/kg feedingstuff for up to 13 weeks, no residues were detected in muscle, levels up to 0.11 mg/kg were found in liver and up to 0.04 mg/kg in other tissues (limit of detection : 0.02 mg/kg). After a 24 h withdrawal period, traces of residues (0.04 mg/kg) were still detected in liver only.

Short- and long-term toxicity studies on laboratory animals were carried out on MCP and MBQ. Both compounds showed low acute toxicity (oral LD_{50} in the rat higher than 16 g/kg b.w. for MCP and 3 g/kg b.w. for MBQ). From two-year oral studies in rats and dogs and a reproduction study on three generations in the rat, the no-effect levels for MCP were respectively 30 mg/kg b.w. and higher than 200 and 300 mg/kg b.w. Changes in the testes were observed in the rat at dosages higher than 30 mg/kg b.w. For MBQ, the no-effect level in the rat, established from a two-year oral study and a reproduction study on three generations, was higher than 200 mg/kg body weight. No mutagenic activity for MCP or MBQ could be detected in <u>in vitro</u> assays with microorganisms. From these data, ADI's for man were estimated at 0.015 mg/kg b.w. for MCP and 2 mg/kg b.w. for MBQ.

For the combined product, Lerbek, LD_{50}'s for rats and chickens are respectively 10 and 4.64 g/kg b.w. In short term studies with

chickens fed at 1, 2, 4 or 10 x the recommended dose level, feed intake and liveweight gain were depressed significantly at the highest dose level by 21 days. No mutagenic activity of Lerbek could be demonstrated in an Ames test.

It is clear from the foregoing that MCP is appreciably absorbed from the digestive tract of rats, dogs and rabbits. Similar pharmacokinetic data are not available for the chicken. MCP appears to undergo little metabolism within the tissues of all the species examined and chickens would be unlikely to behave differently. With MBQ there is little absorption of the product but some metabolism of the small amount that is absorbed does take place within the body. It is the MCP component of Lerbek that gives rise to measurable tissue residues although none are found after 5 days. A 5-day withdrawal period would therefore ensure the absence of any risk to the consumer.

2. In experiments with soils fertilized with poultry litter from chickens receiving MCP in their diet at levels up to 275 mg/kg, residues of MCP in grass decreased from 7 mg/kg to less than 0.1 mg/kg after 26 months. Over this period of time MCP concentrations in the soil fell by two thirds. Studies on lucerne (Medicago sativa), ryegrass (Lolium perenne), lettuce (Lactuca sativa), carrots (Daucus carota) and dwarf beans (Phaseolus vulgaris) grown in soils intimately mixed with Lerbek (at a level considered to reflect the use of poultry manure from birds fed the product) and harvested 3-4 months later have shown that MCP residues in these crops did not exceed 0.2 mg/kg. These values in general agree with the residues observed in a considerable range of vegetables grown in

soils fertilized before planting with 5 tonnes/ha chicken litter containing 124 or 258 mg MCP/kg. After a 3-4 month period of growth, residues were undetectable with the exception of cabbage (Brassica oleracea) and corn fodder (Zea mais), where they amounted 1.1 and 0.2-0.6 mg/kg respectively. In no instance was any phytotoxicity observed.

The presence of MCP in forages is of no consequence for cattle. Studies on beef cattle fed a predominantly hay diet containing from 5 to 50 mg MCP/kg have shown that MCP residues in muscle tissues never exceeded 0.2 mg/kg. In Holstein cows fed diets containing respectively 3 and 10 mg MCP/kg there were no residues of MCP detectable in the milk. With 30 and 100 mg MCP/kg, MCP residues averaged 0.07 and 0.25 mg/l in milk. No residues were detected after a 36 hour withdrawal period.

Lerbek has no effect on soil nitrifying bacteria; in anaerobic sludge there appears to be a small stimulating effect of the product on methanogenic bacteria. Lerbek has no methane-inhibiting influence on rumen contents. Neither of the components of Lerbek is very soluble in water; solubility values are 40 mg/l for MCP and 0.4 mg/l for MBQ. The LC_{50}'s of MCP and MBQ for a number of aquatic organisms are 7 mg/l and 1 mg/l respectively. The foregoing data suggest that contamination of the environment is unlikely.

3. In the light of the available information, the Committee is of the opinion that the use of Lerbek in feedingstuffs for chickens, at a use-level of 110 mg active ingredient/kg, should be maintained subject to a withdrawal period of not less than five days before slaughter.

B. Turkeys

The Committee proposes to express its opinion when data on metabolism of Lerbek in turkeys, its residues and excreted products become available.

REFERENCES

Dossiers Dow Chemicals.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF HALOFUGINONE IN FEEDINGSTUFFS FOR TURKEYS

Opinion expressed 17 November 1982

TERMS OF REFERENCE (October 1981)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of halofuginone as a coccidiostat under the conditions proposed for feedingstuffs for turkeys (see Background) result in the presence of residues in the tissues and organs of the animal? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

2. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

3. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by the thirty-eighth Commission Directive of 16 July 1981 (2), Member

(1) OJ No L 270 of 14.12.1970, p. 1
(2) OJ No L 231 of 15.08.1981, p. 30

States are authorized by way of derogation to use halofuginone under the following conditions set out in Annex II, Section B of the Directive :

Species of animal : chickens for fattening.
Minimum and maximum content in complete feedingstuff : 2-3 ppm (mg/kg).
Other provisions : use prohibited at least five days before slaughter.

An extension of the use of halofuginone under the following conditions has been proposed :

Species of animal : turkeys for fattening.
Minimum and maximum content in complete feedingstuff : 2-3 ppm (mg/kg).
Other provisions : use prohibited at least seven days before slaughter.

OPINION OF THE COMMITTEE

1. Halofuginone excretion was studied after administration for 15 days to turkeys of a diet containing 3 mg of halofuginone (spiked with ^{14}C-labelled halofuginone on the 15th day)/kg feedingstuff. Five days after the administration of the final dose there was a slight residual radioactivity in the liver, kidneys, digestive tract and carcase. Expressed in mg of halofuginone per kg of tissue or organ, the residues ranged from 0.005 to 0.053 (detection limit : 0.005 mg/kg). Radioactivity in the bile was much higher (around 0.3 mg of halofuginone/kg), which indicates that excretion is mainly via the enterohepatic cycle. Studies on the nature of the residues have shown that their principal constituents are unchanged halofuginone and a halofuginone conjugate.

In turkeys fed for 16 weeks with a supplemented feedingstuff (3 mg halofuginone/kg) the residues determined by HPLC (detection limit : 0.02 mg halofuginone/kg) were less than 0.1 mg/kg in all tissues immediately after treatment. After three days of withdrawal they were at the analytical detection limit.

2. A comparison of the carbon 14 studies using chickens and turkeys revealed a significant positive correlation between the tissue concentrations and elimination kinetics of halofuginone on the two species.

Because the metabolism is similar it may be assumed that the data concerning the biodegradation of halofuginone and halofuginone metabolites from chicken droppings in soil and water are also valid for the same substances derived from turkey droppings. The data concerning chickens, originally obtained in 1979, have been amplified with the results of several other studies requested by the Scientific Committee, and now dispelled any doubts concerning the effects of halofuginone on the environment (3).

Droppings of chickens fed orally for 15 days with a dose of halofuginone labelled with ^{14}C (in the quinazolinone nucleus), at a rate of 0.3 mg/kg bodyweight/day, were used for a number of experiments. The droppings were mixed with samples of river water and kept in darkness at 25°C. Tests on the radioactivity of the filtrate showed that the activity never exceeded 76% of the total radioactivity and that the activity associated with unchanged halofuginone, which

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(3) Commission of the European Communities. Reports of the Scientific Committee for Animal Nutrition, Second Series (1980) EUR 6918, pp. 11-13.

was initially 20%, had dropped to 4-5% of its original value in the space of 32 weeks. Several breakdown products less polar than halofuginone were also detected in the filtrate and some of them identified.

The same chicken droppings, mixed with two types of soil, either in controlled laboratory conditions or in field plots, showed that halofuginone and halofuginone metabolites are broken down slowly, giving off $^{14}CO_2$. Within 16 weeks the total radioactivity is reduced to less than 30% of its initial value and any quantities migrating to a soil depth exceeding 5 cm do so in very small quantities and only after 32 weeks. This indicates that the risk of leaching are insignificant. The half-life of halofuginone in soil is of the order of 43 days.

In crops of sugar beets, potatoes and carrots grown in pots, no transfer of radioactivity from the soil (treated with 10 tonnes of droppings/ha) to the plants was observed (detection limit of radioactivity expressed as halofuginone : 0.004 mg/kg), except in carrot tops (0.007 mg/kg). The presence of residues of halofuginone in soil therefore presents no risk for the crops.

3. In view of the foregoing, the Committee has no objections to the use of halofuginone under the conditions of use proposed for turkeys. However, it is unable to issue a final opinion before the results of the additional mutagenicity tests requested previously are available.

REFERENCES

Dossiers Roussel Uclaf and Huntingdon Research Centre.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF VIRGINIAMYCIN IN FEEDINGSTUFFS FOR LAYING HENS

Opinion expressed 17 November 1982

TERMS OF REFERENCE (October 1981)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Has the use of the antibiotic virginiamycin under the conditions proposed for feedingstuffs for laying hens (see Background) a significant effect on egg production?

2. Does this use under the proposed conditions result in the presence of residues in eggs? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

3. In the light of the answers to the above questions, are the poposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by the thirty-eighth Commission Directive of 16 July 1981 (2), the use of virginiamycin is authorized at Community level under the conditions set

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 231, 15.08.1981, p. 30

out as follows in Annex I, Section A, to the Directive :

Species of animal	Minimum content	Maximum content
	ppm (mg/kg) of complete feedingstuff	
Turkeys (up to 26 weeks)	5	20
Other poultry, excluding ducks, geese, laying hens and pigeons (up to 16 weeks)	5	20
Piglets (up to 4 months)	5	50
Pigs (up to 6 months)	5	20
Calves (up to 16 weeks)	5	50 (*)
Calves (up to 6 months)	5	20
		80 (**)

(*) authorized by derogation up to 30 June 1982 (Annex II)
(**) milk replacers

An extension of the use of virginiamycin under the following conditions has been proposed :

Species of animal : laying hens.
Minimum and maximum content in complete feedingstuffs : 10-40 ppm (mg/kg).

OPINION OF THE COMMITTEE

1. Many experiments were performed regarding the use of virginiamycin in feedingstuffs for laying and breeding hens. A study of the dose/response relationship with the range of proposed doses (10, 20 and 40 mg/kg complete feedingstuff) was conducted on the basis of six trials involving 17 024 layers including 4 100 breeding hens.

The number of non-breeding layers on which the 40 mg/kg dose was tested was very small, however.

The results showed that the addition of virginiamycin to feedingstuffs has an appreciable effect on egg production and on feed conversion. The dose/response relationship, however, does not appear to be linear. Statistical analysis of the data revealed that, taking into account the test conditions for each trial, the average increase in egg production compared with the controls are respectively 2.79, 2.65 and 3.91% for doses of 10, 20 and 40 mg/kg of feedingstuffs. At the same doses the average reduction in feed conversion compared with the controls was respectively 1.26, 2.39 and 2.22%.

Because the effects observed varied depending on the dose used, and in view of the fact that the 40 mg/kg dose was tested on an insufficient number of laying hens, the Committee considers that additional trials are required before this dose can be justified. Such trials should extend over one year and involve at least 800 layers.

2. Virginiamycin residues in eggs were sought by microbiological methods. In an initial series of research no residues were found in eggs at the detection limit of 0.25 mg/kg, even when virginiamycin was administered to laying hens in doses of 500 mg/kg of feedingstuff. By subsequently applying a microbiological method which was ten times more sensitive it was possible to establish that the addition of 20 and 80 mg of virginiamycin per kg of complete feedingstuff did not give rise to the presence of residues in eggs (detection limit : 0.02 mg/kg for albumen; 0.02 to 0.05 mg/kg for yolk).

Studies of the metabolism of virginiamycin using molecules labelled with ^{14}C or tritium have been performed on broilers, pigs and rats. It has been shown that the product is very feebly absorbed and that the small quantities of radioactive residues detected in the various tissues and organs after the oral administration of virginiamycin result from the metabolic breakdown of the product into molecules with short carbon chains which have no antibiotic effect. While it is regrettable that a study of the metabolism using labelled molecules was not performed on laying hens, on the basis of the studies on broilers and the microbiological tests it is possible to conclude that the presence of virginiamycin in eggs is not very probable.

3. For the reasons set out above, the Committee is of the opinion that the proposed use of virginiamycin in feedingstuffs for laying hens should not constitute any risks to the consumer. The product is efficacious at doses of 10 to 20 mg/kg of complete feedingstuff. The 40 mg/kg dose should be justified by additional trials lasting one year and involving at least 800 layers.

REFERENCES

Dossiers Smith Kline Ltd.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF CANTHAXANTHIN IN FEEDINGSTUFFS FOR SALMONS AND TROUT

Opinion expressed 14 December 1982

TERMS OF REFERENCE (November 1982)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Is canthaxanthin free of harmful effects when added to feedingstuffs for salmons and trout at a dose-level of 200 mg/kg complete feedingstuff?

2. Does the desired pigmentation of the flesh and skin of the fish necessitate the use of canthaxanthin doses up to 200 mg/kg complete feedingstuff throughout the rearing period?

3. Does the addition of canthaxanthin to feedingstuffs result in significant effects on salmon and trout breeding?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the 40th Commission Directive of 23 June 1982 (2), Member States are authorized to use canthaxanthin in feedingstuffs for poultry with a maximum content of 80 mg/kg complete feedingstuff and in dog and cat food.

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 213, 21.07.1982, p. 22

It was proposed to extend the use of canthaxanthin to feedingstuffs for salmons and trout with a maximum content of 200 mg/kg complete feedingstuff.

The Scientific Committee for Food, consulted by the Commission on the safety of use of canthaxanthin in food, expressed a favorable opinion (Scientific Committee for Food, 1975). The acceptable daily intake for man, estimated by the Joint FAO/WHO Expert Committee on Food Additives, is 25 mg/kg b.w. (WHO, 1975).

OPINION OF THE COMMITTEE

1. Canthaxanthin (4,4'-diketo-B-carotene) belongs to the group of xanthophylls. It is naturally present in an edible mushroom (Cantharellus cinnabarinus), in various fishes (Clupea harengus, Coregonus lavaretus, Crinilabrus tinca, Evynnis japonica, Salmo iridens, Salmo gairdneri, Salmo trutta, Salmo salar, etc.) and shell-fish (Artemis salina, Daphnia magna, etc.), in some algae as well as in the plumage of some birds. Bauernfeind (1981) has published a comprehensive study on this pigment.

   Canthaxanthin added to feed is well tolerated by salmonidae. No apparent harmful effects on behaviour and growth were observed in trout when fed for several months with feedingstuffs supplemented with 450 mg canthaxanthin/kg (Schmidt and Beker, quoted by Auger, 1973).

2. Various studies showed that pigmentation of trout already appears with the addition of 40 mg canthaxanthin/kg feedingstuff. In these con-

ditions, the flesh contains 75-136 micrograms canthaxanthin/100 g after 16 weeks. The fixing by the flesh in the rainbow trout is weak. According to Choubert and Luquet (1979, 1982), the absorption ranges from 5 to 40% depending on the conditions of use. Overpigmentation of the flesh is unlikely to occur.

Schmidt and Beker, quoted by Auger (1973), tested diets containing 190 to 450 mg canthaxanthin/kg feedingstuff for 7 to 31 weeks. The pink tinge of the flesh of the fishes was more sustained and slightly different from that of naturally pigmented fishes. This artificial pigmentation was more stable to heat.

Although there are no physiological, toxicological or organoleptic contra-indications to the proposed use of canthaxanthin in feedingstuffs for salmonidae, the desired pigmentation may be obtained by feeding the trout during the last 3-4 weeks of the rearing period with a ration supplemented with 200 mg canthaxanthin/kg. For the salmon, the addition of 100 mg canthaxanthin/kg complete feedingstuff is sufficient, if the feeding is performed throughout the rearing period.

3. Canthaxanthin promotes the fertility in trout (Hartmann et Meden, 1947; Deufel, 1965, 1975). It has a vitamin A activity in this species (Bauernfeind, 1981).

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF LASALOCID SODIUM IN FEEDINGSTUFFS FOR CHICKENS

Opinion expressed 14 December 1982

TERMS OF REFERENCE (November 1980)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of the coccidiostat lasalocid sodium in feedingstuffs for chickens, under the conditions authorized (see Background), result in the presence of residues in animal products? If so, what is the nature and the amount of these residues? Could these residues be harmful to the consumer?

2. Could the use of this additive induce the development of resistance in bacteria?

3. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

4. In the light of the answers to the above questions, should the conditions of use authorized for this additive be maintained or should they be modified?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the thirty-fourth Commission Directive of 4 September 1980 (2), Member States are authorized by way of derogation to use lasalocid sodium under the following conditions set out in Annex II, Section B, of the Directive:

Species of animal : Chickens for fattening.
Minimum and maximum content in complete feedingstuffs : 75-125 ppm (mg/kg).
Other provisions : use prohibited at least five days before slaughter.

OPINION OF THE COMMITTEE

1. In studies with ^{14}C-labelled lasalocid in chicken about 95% of the dose was found in the excreta where three metabolites were identified as hydrolysis breakdown products. The remaining 5% appeared as residues in liver, kidneys, skin, fat and muscle. With the exception of two metabolites in the liver none other has been identified. After oral administration to chickens the highest residue levels were found in the liver when radioactivity measurements were used, but in skin and fat when microbiological assays were employed.

   In chickens fed for three weeks ^{14}C-lasalocid at 75 and 125 mg/kg

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 251, 24.09.1980, p. 17

feedingstuff, the residues determined by radioactivity measurements were immediately after treatment 4 to 12 mg/kg in the liver and 0.6 to 2.5 mg/kg in other tissues. After a 5-day withdrawal period these residues had decreased to 0.7-1.25 mg/kg in the liver and to $\leq$ 0.13 mg/kg in the other tissues. After 39 days or eight weeks of the same diets, the residues determined by microbiological assay and expressed as lasalocid were of the order of 0.1 mg/kg in liver and kidneys, 0.4 mg/kg in skin and fat and 0.02 mg/kg in muscles immediately after the treatment. After a one-day withdrawal period, traces of residues were detected in skin and fat only. The difference between residue levels obtained by radioactivity measurements and microbiological assay can be explained as a consequence of the metabolism of lasalocid in the liver into non-microbiologically active ^{14}C-labelled compounds.

Lasalocid was investigated in short- and long-term toxicological studies on laboratory animals. The acute oral toxicity for the rat was about 0.1 g/kg b.w. In a 2 1/2 year rat feeding study the no-effect level was 10 mg/kg feedingstuff (about 0.5 mg/kg b.w.). At higher dosages changes in haematology, biochemistry and organ weights were observed. In a 2 year oral study in dogs the no-effect level was 35 mg/kg feedingstuff. A 3 generation reproduction study in the rat lead to the same value. In <u>in vitro</u> test with microorganisms no mutagenic activity could be detected. From the long-term study in rats an ADI for man of 0.005 mg/kg b.w. was established.

In the light of this information, the use of lasalocid under the conditions authorized should not constitute any risks to the consumer.

2. The product is inactive against Gram-negative bacteria, especially E. Coli. In Gram-positive bacteria (Streptococcus and Staphylococcus), occasionally the sensitivity decreased very slightly after exposure to the antibiotic. However, none of the bacterial strains tested showed cross-resistance to antibiotics used in therapeutics, even when a transient resistance to lasalocid sodium developed. The fact that lasalocid is inactive against Gram-negative bacteria means that this product cannot induce the selection of enterobacteria carrying R-factor.

   In the light of this information, it would appear that the use of lasalocid sodium does not induce significant development of bacterial resistance.

3. At dose levels of 75-125 mg/kg of feed, the amount of lasalocid in chicken excreta varies from 6 to 2 mg/kg, depending on the fattening period and the moisture content. In chicken excreta, kept in aerobic conditions at 32°C and 85% humidity, degradation attains 50% in 48 hours and 75% in 15 days. In anaerobic conditions degradation is slight.

   In litters used for several successive batches of fattening chickens treated with lasalocid, the concentration never exceeds 2-6 mg/kg; untreated chickens reared on such litters show no residues of lasalocid in the tissues.

   A number of studies show that lasalocid in the soil (incorporated in chicken excreta or added as such, even in concentrations considerably in excess of those encountered in practice) is rapidly broken down by chemical and microbiological processes; breakdown is complete within 2-3 weeks depending on the type of soil. Lasalocid incorporated in

excreta or in the soil migrates into water where it is broken down : light, heat and alkalinity greatly accelerate degradation, and in aqueous extracts of faecal matter the rate of degradation exceeds 95% in 4 hours. Even at concentrations of 7.5-22.5 g/hectare, equivalent to 1-5 tonnes of excreta per hectare, lasalocid has no phytotoxic effect and does not affect the growth of plants (Zea mays, Hordeum vulgare, Glycine max, Lycopersicon esculatum, Colocynthis citrullus). It has no pesticide effect. It is only slightly toxic towards aquatic organisms (Daphnia magna, Carassius auratus, Lepomis macrochirus). (The no-effect level is 1.0 mg/litre). Concentrations of lasalocid capable of inhibiting methanogenesis are higher than those encountered in chicken excreta. The rapid degradation of lasalocid in the soil, and above all in aqueous extracts of droppings, precludes any activity against nitrifying bacteria in the soil.

In the light of this information, it would appear that the excreted products, derived from the additive, are not prejudicial to the environment.

4. In the light of the available information, the Committee is of the opinion that the use of lasalocid sodium in feedingstuffs for chickens, at use level of 75-125 mg/kg (ppm), should be maintained subject to a withdrawal period of not less than five days before slaughter.

REFERENCES

Dossiers Hoffmann-La Roche.

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF FORMALDEHYDE IN FEEDINGSTUFFS FOR PIGLETS

Opinion expressed 20 April 1983

TERMS OF REFERENCE (October 1981)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Could the use of formaldehyde as preservative under the conditions proposed for skimmed milk (see Background) be harmful to piglets?

2. Does this use under the conditions proposed for piglets result in the presence of residues in tissues and organs of the animal? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

3. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by the thirty-eighth Commission Directive of 16 July 1981 (2), Member States are authorized by way of derogation to use formaldehyde without

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 231, 15.08.1981, p. 30

specific conditions.

The admission of formaldehyde at Community level under the following conditions has been proposed :

Species of animal : piglets.
Feedingstuff : skimmed milk.
Maximum content : 0.06% (=0.15% of formalin containing 40% of formaldehyde).

OPINION OF THE COMMITTEE

1. Formaldehyde's antimicrobial properties are well known. It is a very reactive compound, in particular with proteins, with which it combines, inter alia via the primary amine radicals.

   Depending on the dose-level, time lapse, pH and temperature of the medium, formaldehyde may be present in feedingstuffs in the free state or reversibly bound in the form of methylols and/or irreversibly bound by the formation of non-hydrolysable compounds. The proportion of irreversibly-bound formaldehyde increases with the dose-level, while protein solubility and sensitivity to the action of proteolytic enzymes decreases, together with the quantity and bio-availability of lysine (formation of ε -N-methyllysine) (Tome et al., 1979).

   The metabolism of formaldehyde in monogastric animals is not fully known. Formaldehyde is oxidized to a slight degree to CO_2 and water in various tissues of the rat. (Koivusalo, 1956; Neely, 1964). Free formaldehyde may react by dismutation in the hepatocytes and produce

formic acid and methanol as a result of the action of alcohol dehydrogenase (Abeles and Lee, 1960; Gupta, 1970). Free formaldehyde may also condense with tetrahydrofolic acid to produce methyl tetrahydrofolate. It is therefore possible for formaldehyde, released in the digestive system, to be partly metabolized in monogastric animals. Additional studies, using ^{14}C labelled molecules, are needed to elucidate the metabolism of formaldehyde in pigs.

Considerable experimental evidence from feeding all-milk diets artificially to piglets and a milk supplement to sucking pigs and to bacon pigs (weight range 20-90 kg) indicates that formaldehyde in whole or skimmed milk up to a level of 0.04% (=0.1% formalin containing 40% formaldehyde) is without adverse effects on the animals, as judged by nutritional parameters and carcase characteristics. Reduced appetance has been reported at a dose-level of 0.06% but with little effect on overall performance.

When pigs were given part of their feed as meal and part as 3.5, 4.5 or 5.75 l/day of formaldehyde-treated (0.04%) skim milk, over a period of about 3 months, comparisons of samples of tissues from control and treated pigs taken at slaughter gave no evidence of increased amounts of formaldehyde attributable to the treated skim milk. The mean concentration of formaldehyde in the tissues of the control animals and the animals given formaldehyde-treated skim milk was about 20 mg/kg tissue, with a limit of accuracy of 10 mg/kg tissue for the method of analysis (Jordan and Weatherup 1976, Florence and Miller 1981, Mitchell 1981).

Formaldehyde may be found in various animal tissues used as human food but metabolic studies are difficult because of its reactivity with

food constituents, particularly proteins which give rise to bound forms presenting analytical problems. However, information on the toxicological effects of formaldehyde can be derived from a report of the joint FAO/WHO Expert Committee on Food Additives concerning hexamethylene-tetramine, the toxicological effects of which are stated to be due to the liberation of formaldehyde. On the basis of long-term studies with rodents and dogs an ADI for man of 0-0.15 mg/kg has been established.

3. On the basis of the foregoing information, bearing in mind the limitations of the analytical method, the Committee is of the opinion that the proposed conditions of use are acceptable. However, a number of precautions must be taken when adding formalin containing 40% of formaldehyde to skimmed milk. Formaldehyde solutions are irritant for the eyes, the skin and the respiratory tract.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF COPPER COMPOUNDS IN FEEDINGSSTUFFS FOR PIGS

Opinion expressed 1 June 1983

TERMS OF REFERENCE (June 1982)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions : could copper compounds in feedingstuffs for swine be admitted without prejudice in contents higher than 125 mg/kg complete feedingstuffs, subject to limitations according to the age or destination of the animals, as indicated in the proposals (a), (b) and (c) hereafter?

	Maximum content (mg Cu/kg complete feedingstuffs)
Proposal (a)	
piglets up to 13 weeks	200
swine, with the exception of swine for breeding	100
swine for breeding	50
Proposal (b)	
piglets up to 16 weeks	200
swine up to 6 months	125
swine of more than 6 months	50
Proposal (c)	
swine up to 4 months	200

BACKGROUND

In its opinion delivered on 15 April 1982 (Commission of the European Communities 1983), the Scientific Committee recommended that the maximum content of copper in complete feedingstuffs for swine does not exceed 125 mg/kg. This limitation was justified by reasons of protection of the environment.

Proposals (a), (b) and (c) mentioned above were established taking into consideration both the particularly beneficial effects of copper on the growth of young animals and the protection of the environment. These proposals were presented to the Commission as alternative solutions for the recommendation of the Scientific Committee of 15 April 1982.

OPINION OF THE COMMITTEE

The Committee compared proposals (a), (b) and (c) with the proposal on which it delivered an opinion on 15 April 1982, in terms of the consumption of feed in the various types of pig farming in the Community.

Allowing that the quantity of copper excreted by a pig is proportional to the quantity ingested (Brajon and al. 1980, C.L.O. 1978), proposals (a), (b) and (c) are acceptable from the environmental point of view in certain farming conditions. The duration of the breeding cycle and the spacing out of slaughter in relation to the weight or age of the animal are determining factors. The variability of these factors, together with the gradation of the quantities of copper administered according to the age of the animal or the purpose for which it is being raised, though does not favour the adoption generally of these proposals. The significant variation that this would entail in the quantities of copper excreted could in several cases be detrimental to the objective of protecting the environment.

Furthermore, the change in diet for 13-week-old piglets under proposal (a), or for 4-month-old piglets under proposals (b) and (c), which is common practice in certain types of pig farming, is unsuitable for others where a uniform system of feeding is carried out. The exclusive rearing of piglets fed as in proposals (a), (b) and (c) would also help undermine the above mentioned objective since it would involve the ingestion of quantities of copper 1.6 times greater than those proposed by the Committee in 1982.

Moreover, research into the effects of heavy metals on the selection of bacteria resistant to antibiotics has shown that increasing the quantity of copper from 125 up to 200 mg/kg in pig feed favoured the selection of strains of E. Coli resistant to chloramphenicol (Gedek 1981).

In the light of these findings, the Committee considers that proposals (a), (b) or (c) do not constitute alternative solutions to its proposal of 1982, namely that the maximum content of copper in complete feeding-stuffs for piglets and swine should not exceed 125 mg/kg. However, because of differences in the composition and the physical-chemical nature of soils and of the population density of swine as influenced by housing and types of pig farming, alternative solutions could be envisaged, in particular by allowing the use of copper levels somewhat higher for some categories of swine and rearing times.

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REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF AVOPARCIN IN FEEDINGSTUFFS FOR CALVES AND FATTENING CATTLE

Opinion expressed 1 June 1983

TERMS OF REFERENCE (April 1982, expanded in July 1982)

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions :

1. Does the use of the antibiotic avoparcin under the conditions proposed for milk replacers for calves and feedingstuffs for fattening cattle (see Background) have significant effects on the animal's growth?

2. Does its use under the proposed conditions result in the presence of residues in tissues and organs of the animal? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

3. Could its use affect the development of resistance in bacteria.

4. Could the excreted products, derived from the additive, be prejudicial to the environment? If so, what is the nature of the risks?

5. In the light of the answers to the above questions, are the proposed conditions of use acceptable?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs (1), as last amended by the thirty-ninth Commission Directive of 15 January 1982 (2), the use of avoparcin is authorized at Community level under the conditions set out in Annex I, Section A, of the Directive :

Species of animal	Minimum content	Maximum content
	ppm (mg/kg) of complete feedingstuffs	
Chickens for fattening	7.5	15
Piglets, up to 4 months	10	40
Pigs, 4 to 6 months	5	20
Turkeys, up to 16 weeks	10	20

The Scientific Committee for Animal Nutrition expressed a favourable opinion on these conditions of use (3,4).

It is proposed that the authorization of use of this additive be extended by the following provisions :

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 42, 13.02.1982, p. 16
(3) Commission of the European Communities. Reports of the Scientific Committee for Animal Nutrition. Second Series (1980) EUR 6918.
(4) Report of the Scientific Committee for Animal Nutrition on the use of avoparcin in feedingstuffs for turkeys, 7 October 1981 (unpublished).

Milk replacers for calves (up to 6 months), minimum and maximum content : 40-80 ppm (mg/kg).
Complementary feedingstuffs for fattening cattle, minimum and maximum content expressed on the basis of complete feedingstuffs : 15-45 ppm (mg/kg).

OPINION OF THE COMMITTEE

1. The use of avoparcin in milk replacers for calves (up to 6 months old) was the subject of numerous experiments. With pre-ruminant calves a study was carried out on the dose/effect relationship (0, 20, 40, 80, 120 mg/kg of milk replacer in powder form), on the basis of 9 trials involving a total of 420 animals. The number of calves on which the dose of 120 mg/kg was tested was, however, too small to permit a reliable assessment.

   Examination of the results reveals that the dose of 80 mg/kg is not justified because it does not provide results which differ statistically from those provided by 40 mg/kg. Statistical analysis involving the calves treated with 20 mg/kg (Duncan test), however, showed a difference in the efficacy of avoparcin which was statistically significant compared with the controls (0 mg/kg). The results obtained showed that the addition of avoparcin has appreciable effects on daily weight gain and on the feed conversion ratio. However, the dose/effect relationship does not appear to be a linear one.

   According to these data, avoparcin is effective in milk replacers at dose-levels of 20 and 40 mg/kg.

   The use of avoparcin in the feed for beef cattle with a partially or fully functioning rumen was the subject of a total of 32 experiments performed on 3 365 animals. A study of the dose/effect relationship

was carried out with the following doses (mg/kg of feedingstuff) : 0, 10, 15, 30, 45, 60 and 90. The research was performed on cattle of different breeds and initial weights, for varying periods, and with different energy levels, feeding patterns and bioclimatic conditions. The results obtained showed that the addition of avoparcin has appreciable effects on average daily weight gain and on feeding efficiency at doses of between 15 and 45 mg/kg of feedingstuff, even though the dose/effect ratio is not linear either in the case of weight gain or in the case of feeding efficiency.

In the light of these figures, the minimum and maximum content proposed for complementary feedingstuffs for fattening cattle (15 and 45 mg/kg of compound feedingstuff) are thus appropriate. In order to prevent incorrect use of avoparcin in ruminant cattle receiving complementary feedingstuffs, however, it is considered useful to establish a maximum daily dose per animal based on live weight. It is accepted that feed intake of ruminant cattle does not increase in direct proportion to body weight. This necessitates an adjustment of the amount of additive in the ration. In view of the foregoing, the maximum amount in the daily ration should not exceed 90 mg (constant value) + 65 mg/100 kg live weight. The values obtained according to this formula are as follows :

Weight of the animal (kg)	Average daily consumption of feed (kg)	mg avoparcin/ head/day (90 mg + 65 mg/100 kg live weight)	ppm equivalent (mg avoparcin/kg complete feedingstuff)
100	3.4	155.00	45.00
150	4.4	187.50	42.60
200	5.6	220.00	39.30
250	6.7	252.50	37.70
300	7.6	285.00	37.50
350	8.3	317.50	38.30
400	9.0	350.00	38.90
450	9.6	382.50	39.20
500	10.4	415.00	39.90
550	10.5	447.50	42.60
600	10.9	480.00	44.00

2. In its opinion given on 11 July 1979 (Commission of the European Communities, 1980) the Committee stated that avoparcin added to feed was virtually unabsorbed by the digestive system in rats, chickens and pigs and that its use in feed for chickens and pigs did not produce any detectable residues in the tissues.

Studies performed on calves, steers and bulls under the proposed conditions and at higher doses and with the use of molecules labelled with ^{14}C lead to the same conclusions.

In veal calves which had received feedingstuffs with 40, 80 and 400 mg of avoparcin per kg for 10 to 19 weeks no residues detectable by microbiological assay (detection limit : 0.20 to 0.25 mg/kg) and no antibiotic activity were found in the muscles, liver, kidneys or subcutaneous fat. The withdrawal periods before slaughter were respectively 0, 1 and 3 days.

In steers to which 2 mg/kg body weight of avoparcin labelled with ^{14}C had been administered orally for eight consecutive days, and which had been slaughtered two hours after the last administration, the radioactivity administered was recovered quantitatively (88% in faeces, 20% in the rumen, 4.5% in the contents of the digestive system, 0.1% in the urine and 0.5% in the wash water from the metabolism cages). The blood, liver, muscles and fatty tissues were free of radioactive residues (detection limit expressed in terms of avoparcin : 0.05 mg/kg); there were traces in the kidneys (0.05 to 0.06 mg/kg).

Other studies on steers which had received feed containing 97 mg of avoparcin per kg for 56 days and bulls which had received feed containing 200 mg of avoparcin per kg for five months showed that the blood, liver, kidneys, muscles and fat of the animals were free of residues which could be detected either by microbiological assay (detection limit : 0.25 to 0.50 mg/kg according to substrate) or by thin layer chromatography (detection limit : 0.1 mg/kg).

Since the proposed uses of avoparcin do not cause detectable residues in edible products, they do not entail any risks for the consumer.

The microbiological effects of the addition of avoparcin to feed for calves and fattening cattle were the subject of several experiments.

In a ten-week experiment on four groups of nine calves each, which were fed doses of 0, 40, 80 and 400 mg/kg of feedingstuff respectively, a marked reduction in gram-positive bacteria (enterococci and lactobacilli) was observed, depending on the dose, and there was a slight increase, irrespective of the dose, in gram-negative bacteria

(E. coli). All the calves in the experiment were fed a combined antibiotic for three days before the start of the experiment. As could be expected, the E. coli strains (143 strains) isolated at the start of the experiment were mostly multi-resistant, whereas those isolated at the end of the experiment (142 strains) showed a considerable reduction in the number of multi-resistant strains.

In an experiment lasting six weeks which included among others two groups of eight yearling steers, one given no avoparcin and the other 60 mg avoparcin/kg of feedingstuff, the number of intestinal bacteria (E. coli and enterococci) did not seem to be affected by the use of avoparcin. The resistance pattern in general remained unchanged throughout the experiment.

The effects of administering avoparcin to calves on certain salmonellae strains was also studied. In a six-week experiment which included four groups of ten calves, two of the groups were infected with S. thypimurium and fed diets containg 0 and 80 mg of avoparcin/kg of feedingstuff. The infected animals excreted S. typhimurium between the seventh and the thirty-fifth day independently of the administration of avoparcin. This additive did not lead to colonization by salmonellae.

In another six-week experiment four groups of nine calves received doses of 0, 20 , 40 and 80 mg of avoparcin/kg of feedingstuff. At the end of the experiment two calves had died and 20 of the remaining 34 were found to be infected with S. dublin. Avoparcin was not observed to have any effect.

These residues show that the use of avoparcin as an additive in feed for calves and steers does not lead to the development of resistance in intestinal bacteria and does not influence the effects of S. typhimurium and S. dublin on the organism of these animal species.

4. In pre-ruminant calves and fattening cattle most of the avoparcin added to the feed is eliminated as such in the faeces. In this environment avoparcin breaks down fairly rapidly. Its half-life has been estimated on the basis of antibiotic activity at 8 to 16 days at temperature of 28 and 37°C.

The use of faeces of pre-ruminant calves or fattening cattle containing avoparcin as manure has no apparent phytotoxic effects or any disadvantages for aquatic organisms. The incorporation of 22 tonnes/ha of faeces containing 15 or 150 mg of avoparcin per kg in the soil does not affect the microbial nitrification process. At 150 mg/kg (=10 times the quantity excreted by the animal after administration of 40 mg/kg of feed) there is even a stimulant effect on the production of nitrates. Methanogenesis is not affected by the faeces of pre-ruminant calves of cattle receiving feed containing 40 or 45 mg of avoparcin/kg.

These observations confirm those reported for pigs. They lead to the conclusion that under the proposed conditions of use avoparcin cannot have harmful effects on the environment.

5. In the light of the figures available, the Committee is of the opinion that, for the sake of effectiveness the minimum and maximum avoparcin contents of milk replacers for calves should be reduced to 20 and 40

mg/kg. As regards complementary feedingstuffs for fattening cattle, the Committee takes the view that the minimum and maximum contents proposed (15 and 45 mg/kg of complete feedingstuff) are acceptable. However, to avoid incorrect use of the additive for ruminating cattle the following provision should be added concerning doses : "For ruminant cattle receiving complementary feedingstuffs the maximum dose in the daily ration shall be adjusted so as not to exceed 90 mg + 65 mg/100 kg live weight".

If used under these conditions avoparcin added to milk replacer feeds for calves and complementary feedingstuffs for fattening cattle involves no risks either for the consumer or for the environment.

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