REPORTS OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION

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Reports of the Scientific Committee for Animal Nutrition

(Eighth series - 1992)

DOCUMENT

Foreword

The Scientific Committee for Animal Nutrition (SCAN) was created by Commission Decision 76/791/EEC of 24 September 1976[1] in order to provide the Commission with informed opinions on scientific and technical matters related to animal nutrition and related aspects.

The members of the Scientific Committee for Animal Nutrition are independent and highly qualified scientists in the relevant fields of veterinary and human medicine, animal nutrition, food and feed sciences and environmental protection. The president is elected by its members and the secretariat is provided by the Commission.

The SCAN expresses its opinions on answers to specific questions from the Commission. These are published by the Commission[2]. The opinions expressed reflect the present state of knowledge concerning aspects of animal health and nutrition and other related matters in regard to Community legislation, and in particular:

- Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs[3] as ammended by Council Directive 84/587/EEC of 29 November 1984[4],

- Council Directive 87/153/EEC of 16 February 1987 fixing guidelines for the assessment of additives in animal nutrition[5],

- Council Directive 82/471/EEC of 30 June 1982 concerning certain products used in animal nutrition[6],

(1) O.J. No. L279 (09.10.1976) p. 35.
(2) See cumulative index in pages 87-95 of this report.
(3) O.J. No. L 270 (14.12.1970), p. 1.
(4) O.J. No. L 319 (08.12.1984), p. 13.
(5) O.J. No. L 64 (07.03.1987), p. 19.
(6) O.J. No. L 213 (21.07.1982), p. 27.

- Council Directive 83/228/EEC of 18 April 1983 on the fixing of guidelines for the assessment of certain products used in animal nutrition[7],

- Council Directive 74/63/EEC of 17 December 1973[8] on the fixing of maximum permitted levels for undesirable substances and products in feedingstuffs as amended by Council Directive 86/354/CEE[9].

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(7) O.J. No. L 126 (13.05.1983), p. 23.
(8) O.J. No. L 38 (11.02.1974), p. 31.
(9) O.J. No. L 212 (02.08.1986), p. 27.

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(1) Commission Decision 76/791/EEC (24.09.1976). O.J. No. L 279, 09.10.1976. p. 35.
(2) Appointed by Commission Decission 90/C 115/04 (O.J. No. C 115, 09.05.90, p. 5.
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(6) Chairman until expiry of his term of office on 08.07.1986.

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(1) Council Directive 87/153/EEC, O.J. No. L64 (07.03.87) p.19.

Report of the Scientific Committee for Animal Nutrition on the use of lasalocid sodium in feedingstuffs for finishing cattle. (Opinion expressed : 27 July 1990).

Terms of reference (February 1984)

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

1. Has the use of lasalocid sodium under the conditions proposed for feedingstuffs for finishing cattle significant effects on animal growth?

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

4. Could the excreted products, derived from the additive, be prejudicial to the environment?

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 forty fourth Commission Directive of 29.11.1983(2), the

(1) O.J. N° L 270, 14.12.1970, p. 1.
(2) O.J. N°L350, 13.12.1983, p.17.

use of lasalocid sodium is authorized at Community level under the conditions set out as follows in Annex 1, Section D, of the Directive.

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

The Scientific Committee for Animal Nutrition expressed a favourable opinion on this use in its report of 14 December 1982.

Opinion of the Committee

The file submitted to the Committee related to BOVATEC(*), a premix containing 15% lasalocid sodium.

1. Thirty-two trials with lasalocid sodium were conducted between 1978 and 1987 in several countries on some 2500 cattle of various breeds maintained under different husbandry conditions. They showed that lasalocid sodium, at concentrations between 10 mg and 35 mg/kg of complete feedingstuff, favourably influenced growth and improved the feed conversion ratio. The highest concentration employed did not achieve statistically significant greater effects than lower concentrations.

   Evaluation of the efficacy in relation to the energy density of the animal diet showed the existence of an inverse relationship between feed intake and energy value of the ration. However, the addition of lasalocid sodium to low energy rations tended to increase body weight gain without concomitant effect on feed intake. This phenomenon has been observed also with other ionophore antibiotics. Overall it could be shown that lasalocid sodium, at a concentration of 35 mg/kg of complete feedingstuff, improved feed efficiency from 7% to 15% for low energy rations and from 3% to 5% for high energy rations. Grazing trials using lasalocid sodium at doses ranging from 100-300 mg/animal/day showed a greater improvement in daily body weight gain (the only parameter which can be measured in these trials) than feed-lot trials (in these trials 3 parameters can be measured: feed consumption, feed conversion, and mean daily body weight gain).

(*) Registered trade name of Hoffmann-LaRoche

Lasalocid sodium also reduced the incidence of meteorism (bloat) in cattle kept on dried leguminous feed.

Lasalocid sodium at concentrations of 50 mg/kg feedingstuff, altered the volatile fatty acid composition of the rumen contents by increasing significantly the molar proportion of propionic acid, though even concentrations as high as 176 mg/kg feedingstuff did not increase the total volatile fatty acid concentration in rumen.

Although the proposed levels of use ranging from 10–35 mg/kg of complete feedingstuff appear to be efficacious, there is a need to avoid the incorrect use in ruminating cattle of complementary feed containing lasaloid sodium. It would therefore be appropriate to fix a maximum daily dose for each animal according to its body weight because feed consumption during rumination does not increase proportionally with the body weight. The maximum dose of lasalocid sodium in the daily ration should not exceed 50 mg (constant value) plus 55 mg/100 kg body weight (variable value). The table below sets out the appropriate quantities.

Live weight (kg)	Mean daily feed consumption (kg)	lasalocid Na/head/day (50mg+55mg/100kg b.w.) (mg)	Equivalent dose in complete feed (mg/kg)
100	3.4	115	34
150	4.4	132.5	30
200	5.6	160	29
250	6.7	187.5	28
300	7.6	215	28
350	8.3	242.5	29
400	9.0	270	30
450	9.6	297.5	31
500	10.4	325	31
550	10.5	352.5	34
600	10.9	380	35

The use of lasalocid sodium in feedingstuffs for cattle did not affect carcase quality. Organoleptic tests carried out on beef meat showed no difference between samples from control animals and animals which had received 165 mg lasalocid sodium/kg feedingstuff. No adverse effects due to lasalocid sodium administration were noted in target animals such as chickens, laying hens, turkeys, heifers, cows and horses at doses up to 0.6 mg/kg body weight, equivalent to 35 mg/kg of complete feedingstuff. The tolerance of rabbits to repeated administration of lasalocid sodium has not been determined.

2. The oral acute toxicity of lasalocid sodium was about 100 mg/kg body weight in rats and about 150 mg/kg body weight in mice. The substance was not irritating to the skin and eyes of rabbits. A sensitization test in guinea-pigs was negative.

Mutagenicity studies in prokaryotic and eukaryotic organisms as well as *in vitro* assays for several genetic endpoints were all negative. Long-term studies in mice, rats and dogs showed no evidence of any carcinogenicity. The increased incidence of lymphosarcoma observed in the mouse study was within the range noted in historical controls. It was therefore not considered to be indicative of any carcinogenic potential. At high dose levels changes were noted in some haematological and clinico-chemical parameters and in some organ weights. The no-observable effect level (NOEL) in the rat was 0.5 mg/kg body weight. The highest dose of 6 mg/kg body weight in a three-generation-reproduction study in rats induced weight-loss-related effects on male and female fertility but no evidence of teratogenicity. The NOEL in this study was 1.8 mg/kg body weight. The ADI for man was established at 0.005 mg/kg body weight based on the NOEL in a long-term study in rats. Lasalocid sodium had no adverse effects in target and non-target animal species at doses up to 35 mg/kg feed.

The metabolic fate of lasalocid sodium was studied in steers using ^{14}C-labelled lasalocid. Single dose administration produced very low blood radioactivity levels, 89% of the administered radioactivity being recovered in the faeces and 0.18% in the urine after 24 hours. Some 80% of the faecal radioactivity was extractable, divided into

54% unchanged lasalocid and 26% other products, representing at least 5 metabolites. None of the metabolites was present at more than 4.5%. Lasalocid is partly absorbed and then excreted in the bile either as the parent compound or as related metabolites.

Repeated administration of ^{14}C-labelled lasalocid sodium results after three days in steady tissue levels, the main target organ being the liver. Of the labelled liver residues some 82% were extractable and consisted of 15% unchanged lasalocid, 7% identified metabolites (5 in number) and 60% unidentified fragments. The unidentified portion consisted of many products each representing less than 1% of the extractable hepatic radioactivity. Some 15% hepatic radioactivity was non-extractable. Both the extractable and non-extractable fractions were non-mutagenic when tested in the Salmonella reverse mutation test. The biotransformation products identified had no ionophoric properties. No information has been provided on the identity of the various metabolites.

Cattle treated under field conditions with ^{14}C-labelled lasalocid sodium for 2 weeks at a rate of 1.0 mg/kg body weight (approximately 40 mg/kg feedingstuff) had total labelled residues of 6.92 mg/kg tissue in the liver, 0.06 mg/kg tissue in the kidneys, 0.022 mg/kg tissue in the fat and 0.009 mg/kg tissue in muscle when slaughtered within 16 hours after the last dosing. After 9 days withdrawal period the total labelled residues were 0.713 mg/kg tissue in the liver, 0.019 mg/kg tissue in the kidneys and below the limit of detection in fat and muscle (limit of detection 0.0035 mg/kg tissue). The bioavailability of the liver residues was on average 24.4% as determined in the bile of rats fed with liver from steers treated with labelled lasalocid sodium.

Cattle treated for 4 weeks under field conditions with unlabelled lasalocid sodium at a rate of 0.6 mg/kg body weight/day (approximately 33 mg/kg feedingstuff) had liver residues of lasalocid sodium ranging from 0.025-0.539 mg/kg tissue at zero withdrawal period, less than 0.1 mg/kg tissue after a 2-day withdrawal period, 0.035 mg/kg after a 3-day withdrawal period and no detectable residues after a 4-day withdrawal period (limit of detection of HPLC method

0.025 mg/kg tissue). These figures do not include the 82% identified and unidentified metabolites of lasalocid sodium present in the liver but not determined by the HPLC method. For comparison with the total labelled residues determined by analysis of radioactivity these figures should be multiplied by a factor of at least 7.

Extrapolation from the data of the study using radiolabelled lasalocid sodium would yield a total liver residue of approximately 1.2 mg/kg tissue calculated as lasalocid sodium after a 5-day withdrawal period and about 0.71 mg/kg tissue after a 7-day withdrawal period. Extrapolation from the studies under field conditions yields total liver residues, also calculated as lasalocid sodium, of less than 0.18 mg/kg tissue after a 4-day withdrawal period and residues ranging from 0.18-3.7 mg/kg tissue at zero withdrawal period.

3. The Committee has already delivered its opinion on the possible effects of lasalocid sodium on the development of bacterial resistance when the use as additive to feedingstuffs for chickens was being evaluated. Like other polyether antibiotics it is generally effective against Gram-positive bacteria and ineffective against Gram-negative bacteria and also fungi. *In vivo* the substance inhibits specifically most of the lactic acid producing bacteria in the rumen. It has no effect on the development of Salmonella infections and does not prolong the shedding period of Salmonellae.

The ineffectiveness against Gram-negative bacteria, particularly *E.coli*, suggests that selection of enterobacteria carrying R plasmids is unlikely to occur. Enterococci and other Gram-positive bacteria show some degree of variation in resistance to lasalocid sodium but no persistent changes were noted. These effects were not accompanied by lowered sensitivity of the bacterial strains tested to antibacterial substances in common therapeutic use.

4. Lasalocid sodium, when added to feedingstuffs for cattle, is eliminated mainly in the faeces and in small amounts in the urine. Studies with ^{14}C-labelled material show that 50% of the excreted faecal matter is unchanged lasalocid sodium and the remainder at least 5 metabolites. The degradability of the product in cattle

manure (approximate concentrations 10-12 mg/kg untreated manure, 40-48 mg/kg dry matter) has been studied in aqueous systems in relation to pH, light and temperature. Though very stable in dry faeces concentrations fall by 30% after one month in wet faeces kept at 37°C. Degradation varies with temperature after 1 week in slurry pits but always exceeds 50%.

Spreading of dung and liquid manure from treated cattle leads to soil concentrations of about 0.05 mg/kg soil of which about half leaches out. Static and dynamic trials with different types of soil showed considerable removal by leaching, as well as chemical and microbiological degradation over 2-3 weeks.

Lasalocid does not impair soil methanogenesis. The absence of effects on plant development, particularly soya, makes any deleterious effects on nitrifying soil bacteria unlikely.

As stated in the previous report of the Scientific Committee there was no evidence of any phytotoxic effects when tested on growing maize, barley, tomatoes and cucumbers nor does lasalocid sodium affect the germination of cereals and other plant seeds.

Lasalocid sodium is barely toxic to aquatic organisms such as Daphnia, goldfish, moonfish and fresh water algae. The LD_{50} ranges from 2.4 mg/l to 8.0 mg/l for periods of 48-49 hours.

5. CONCLUSIONS

Lasalocid sodium has been shown to be effective for promoting growth and improving feed conversion ratios in cattle during the finishing period at concentrations between 10 mg and 35 mg/kg of complete feedingstuff. There is, however, a need to fix a maximum dose according to the body weight of the animal as set out in the table in this report.

Residues of lasalocid sodium are found essentially in the liver but are low (about 0.25 mg/kg tissue) even at zero withdrawal periods. As the bioavailability is only about 25% these levels are well within the ADI and would not require a withdrawal period. However, as the identity of the residues is not well defined and as these residues, at the levels detected, have no biological activity, the Committee required additional mutagenicity tests with pure lasalocid sodium.

These additional tests establish adequately the absence of a genotoxic potential for the pure substance. Neither the extractable nor the non-extractable liver residues are mutagenic in a prokaryotic assay. Lasalocid sodium does not cause the development of significant bacterial cross-resistance to antibiotics in common therapeutic use.

The excreted products are not prejudicial to the environment and do not interfere with methanogenesis or with nitrification.

In the light of the above findings the Committee is of the opinion that the use of lasalocid sodium is acceptable in complementary feedingstuffs for finishing cattle at concentrations of 10-35 mg/kg but the maximum dose in the daily ration should not exceed the values given in the table in this report.

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Report of the Scientific Committee for Animal Nutrition on the use of Efrotomycin in feedingstuffs for pigs. (Opinion expressed: 27 July 1990).

Terms of reference (April 1986)

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

1. Has the use of the fermentation product efrotomycin at the dosages proposed for feedingstuffs for pigs under the different climatic conditions existing in the European Community significant effects on growth? Which component of this mixture is the active ingredient? Are pigs of both sexes and all age groups equally affected positively?

2. Is this safe for the pig?

3. Can it result in the development of resistance in bacteria to prophylactic or therapeutic preparations, or exert an effect on the persistence of Gram-negative bacteria in the digestive tract of the pig?

4. What is the metabolic fate of efrotomycin in the pig? Does the proposed use result in residues in animal tissues? If so, what is the qualitative and quantitative composition of these residues?

5. Do the toxicological studies allow the conclusion that the proposed use does not present risks
   - for the consumer?
   - for the user?

6. What are the nature and the persistence of excreted products derived from efrotomycin? Can these products be prejudicial to the environment?

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

Background

Efrotomycin was the subject of an application for admission in Annex II, Section A (Antibiotics), of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs(1) under the following conditions of use:

Species of animal: pig.

Minimum and maximum doses: 2-16 mg/kg of complete feedingstuff.

Opinion of the committee

Efrotomycin is an antibiotic belonging to the elfamycin family. It is structurally a N-methylhydroxypyridone glucoside produced by fermentation with *Nocardia lactamdurans*. The granular premix formulation (Producil*), containing 32 g efrotomycin activity/kg, has been proposed for use as a growth promoter in swine rations at a final concentration of 2-16 mg active ingredient/kg. Efrotomycin consists of 3 components: about 65-75% A_1, 1-7% B, and 2.5-10% A_2. The components A_1 and A_2 are E and Z geometric isomers. Although the stability of the premix at 45°C has not been examined specifically, it is stable at 37°C for about 9 months. The feedingstuff containing 16 mg/kg is reasonably stable for over 3 months at constant humidity. 10% of the activity may be lost in 6 months to 1 year.

The microbiological method of analysis does not give reliable results for the components A_2 and B below levels of 16 mg/kg. It, and the HPLC method of analysis, are however reliable for component A_1 down to the level of 4 mg/kg.

(1) O.J. N° L 270 of 14.12.1970, p. 1.
(Producil*) Registered trade name of Merck, Sharp and Dohme.

1. Efficacy was studied in 37 trials with pigs (23 in UK, France, Germany, Italy, Denmark and 14 in USA, Canada, Brazil) involving a total of 3600 animals including 954 controls, and doses from 2-16 mg/kg of complete feedingstuff. The 23 European trials involved 2612 animals and 540 controls. All these trials were conducted with concentrations of copper between 35-180 mg/kg feedingstuff. In 8 trials 5 different additives (tylosin, olaquindox, virginiamycin, carbadox, avoparcin) were used as positive controls. Daily weight gain and feed conversion efficiency were the zootechnical parameters.

   In the data obtained before 1986 the positive controls often showed no growth promoting effect due to inadequate experimental design. This was not noted in the European trials after 1986. The energy and amino acid composition of the feeds are not stated in many of the trials and the stated doses of efrotomycin frequently do not correspond with those determined by feed analysis. In several trials the number of animals used was small.

   For all European trials the improvement in daily growth was very variable at all tested doses. The same variability was observed for feed efficiency. The dose-response curves in these trials are not linear.

   In 22 trials using doses higher than 4 mg efrotomycin/kg of complete feedingstuff neither growth nor feed conversion index were significantly. Furthermore, in some trials there was no difference in weight gain between 2 mg and 4 mg efrotomycin/kg of complete feedingstuff. In general, results appeared to be better during the early growing period (up to 60 days). The concomitant use of Cu supplementation and of castration in the European trials makes it difficult to assess the specific growth promoting effect of efrotomycin. In some trials males show a better growth response.

2. Studies in the target species using efrotomycin at 5x (80 mg/kg feed), 20x (320 mg/kg feed) and 25x (400 mg/kg feed) the recommended inclusion level revealed no toxicologically relevant effects.

Transient diarrhoea and perianal erythema were seen at 400 mg/kg feed. No adverse treatment-related effects on body weight gain or feed consumption were noted at 320 mg/kg feed or less after 28 days. Treatment with efrotomycin did not influence reproduction parameters or offspring.

Treatment of non-target species with doses up to 40 mg/kg feed resulted in lower feed consumption and diarrhoea in the first few days, disappearing within one week in sheep and cattle. In horses decreased feed consumption and lower body weight gains were observed with 16 mg/kg feed. Birds (chicken, turkey, duck, guinea fowl, quail) treated with efrotomycin at 4 mg/kg feed showed generally higher weight gain and feed conversion efficiency. Higher doses were not studied. No data on rabbits were available.

3. Efrotomycin is a narrow-spectrum antibiotic, its activity being mainly due to the component A_1. Although the component A_2 has the same spectrum of antibiotic activity, its presence is too low to make any significant contribution. Component B has no antibiotic activity. Relatively high inhibitory levels of efrotomycin are required *in vitro*. It may be more effective *in vivo*. Little activity was shown against a large number of bacterial isolates from farms except against *Streptococcus suis*, *Pasteurella multocida*, *Clostridium perfringens*, and *Treponema hyodysenteriae*

   There was little evidence of the development of resistance to efrotomycin in *enterococci*, *coliforms*, *Cl. perfringens* and *bacteroides* species. No mutants resistant to efrotomycin developed in *clostridia*.

4. The metabolic studies used the reverse isotope dilution analysis of the substance labelled with ^{14}C at C 7 (limit of detection 0.005-0.01 mg/kg). Tissue residues were assessed by HPLC/UV (limit of detection 0.005 mg/kg) which correlates well with the microbiological assay method.

Balance studies were carried out in young pigs using ^{14}C-labelled efrotomycin A_1 at 16 mg/kg feed. Recovery studies were carried out over 48 hours only which is an undesirably brief period. 60-77% of radioactivity was recovered in the faeces and urine (less than 2%) after 48 hours with an approximate half-life of 1 day. Efrotomycin A_1 is therefore absorbed to a small extent from the gut, the target organ being the liver.

Liver residue amounted to 0.16 mg/kg tissue, kidney residue to 0.04 mg/kg and muscle 0.011 mg/kg. No radioactivity appeared in the fat. Live residue had fallen to 0.018 mg/kg and kidney residues to 0.013 mg/kg within 48 hours, the half-life being 0.5-1 day. Muscle tissue was free from residue after 24 hours. 65-70% of the liver residue was efrotomycin A_1, 12-16% was non-extractable. No efrotomycin B was detected in the liver. Solvent extraction caused analytical interference because of partial decomposition of efrotomycin A_1.

About 66% of the kidney residue was efrotomycin A_1. Urine contained no efrotomycin B, about 30% of the activity being due to efrotomycin A_1. The remainder were polar metabolites. 60% of the faecal radioactivity was efrotomycin A_1 and 20% efrotomycin B, the remainder being breakdown products. The polar urinary and faecal breakdown products were not identified further. Efrotomycin B was therefore not absorbed from the gut.

The rat metabolizes efrotomycin A_1 approximately similarly to the pig but appears to form fewer polar metabolites. Rat liver contains both efrotomycin A_1 and a smaller percentage of polar metabolites than pig liver.

Pig stomach contents partially convert efrotomycin A_1 into efrotomycin B *in vitro* and this conversion may also occur *in vivo*.

Bioavailability was not studied but in view of the low level residues and very short half-life this is not relevant to safety. Similarly, the low level of the residues for polar breakdown makes further identification unnecessary.

5. The toxicological studies involved acute oral and i.p. toxicity in rats and mice. Efrotomycin-Mg-alginate was non-irritant to rabbit skin and eyes. Subchronic toxicity was studied in dogs and rats. Two-year chronic studies in mice and rats produced no evidence of carcinogenic potential. A two-generation reproduction study in rats showed no treatment-related effects on reproductive parameters. Teratogenicity studies in mice and rats produced some evidence of foetotoxicity at high doses tested (2000 mg/kg b.w.) which however caused no maternal toxicity. Mutagenicity was studied in several test systems, both *in vivo* and *in vitro*. The positive result for efrotomycin in one *in vitro* test could not be confirmed in subsequent tests with the same and a new sample of efrotomycin. An additional *in vitro* test for induction of SCEs in cultured CHO cells was negative. An ADI of 0.1 mg/kg b.w. can be established based on the lowest NOEL of 10 mg/kg b.w. found in the subchronic dog study. However the dog is not as sensitive as some livestock animals. Adverse effects were found in pigs at 16 mg/kg b.w.

6. Decay of the active principle in the excreta is slow in aqueous solution in the dark but rapid in sinlight and varies with pH. It is particularly rapid at acid pH but, even at pH 9, some 94% have decayed in sunlight after 10 hours. Days is also slow in sandy and clay soil. Efrotomycin shows little mobility in the soil and there is a theoretical possibility of accumulation unless it is destroyed by photodegradation. Data on biodegradation in sediment/water systems or activated sludge are not available. The partition coefficient indicates that bioconcentration in aquatic and terrestrial organisms is unlikely. The physical properties make dispersion in the environment by evaporation unlikely. Germination trials showed slight phytotoxicity only against maize and no significant inhibition of root and shoot growth in mono- and dicotyledons. Efrotomycin was not toxic to earthworms at concentrations up to 1000 mg/kg soil. A concentration of 31 mg/l had no effect on *Daphnia magna* and 0.13 mg/l did not affect algal growth. The LD_{50} for *Salmo gairdneri* was greater than 100 mg/l. No negative effects were noted regarding nitrification up to 20 mg/kg in the soil. Soil methanogenesis showed a slight reduction and concentrations above 100 mg/l reduced methane production in waste water treatment plants. No tests with faeces containing efrotomycin

and its degradation products were performed. A Stauber Heubach test on the granulate, the premix and a complete pig feed containing 16 mg efrotomycin/kg showed that the premix produced only 1% of the dust generated from the granulate. No efrotomycin was detected in the dust from the pig feed.

7. The zootechnical data relating to the European trials give only weak support to the efficacy claims for efrotomycin when used in conjunction with copper supplementation and castration of males. No data are available to judge whether the different climatic conditions within the European Community affect the growth promoting action of efrotomycin. The US trials are adequate for substantiating the efficacy of efrotomycin in the absence of copper supplementation. Both the A_1 and the B components appear to be more effective in younger animals and best in uncastrated males. Consideration of the various efficacy trials showed that the effective dose was about 4 mg/kg of complete feedingstuff. Studies in the target species have shown that doses up to 16 mg/kg feed are safe for pigs.

   There was no evidence for the development of resistance to prophylactic or therapeutic preparations or of the persistence of Gram-negative bacteria in the digestive tract of the pig nor of any effect on Salmonella shedding. Efrotomycin A_1 is absorbed to a small extent, undergoes biotransformation and is found in the liver at very low levels. The half-life of these residues is about 24 hours. Bioavailability is not relevant for the safety of these residues in view of their low level and short half-life.

   The toxicological profile of efrotomycin has been fully studied, the ADI of 0.1 mg/kg b.w. being based on the dog study. There is an adequate safety margin between the residues in the liver and the ADI. On this basis the use at the level of 4 mg/kg of complete feedingstuff does not present any risks to the consumer. The Stauber-Heubach test confirmed the absence of significant risk from dust inhalation from the premix or complete feedingstuff.

Efrotomycin is photodegradable, shows little mobility in soil and has therefore a theoretical potential for accumulation unless it is destroyed by photodegradation. It does not bioconcentrate nor does it evaporate into the environment. It is not phytotoxic nor toxic to earthworms, *Daphnia*, fish and vegetable forms of aquatic life. It does not affect significantly nitrification or methanogenesis. No data on the effect on meat quality are available.

In the light of the above the Committee is of the opinion that the use of efrotomycin is acceptable at a level of 4 mg active ingredient/kg of complete feedingstuff. The amount of efrotomycin added to the premix or the complete feedingstuff should be stated in terms of efrotomycin A_1, the only component analysed in feed and residues.

REFERENCES

Dossiers supplied by Merck, Sharp and Dohme.

Jacks, T.M., Frazier, E., Judith, F.R., Olson, G. (1988). **Effect of efrotomycin in feed on the quantity, duration and prevalence of shedding and antibacterial susceptibility of Salmonella typhimurium in experimentally infected swine.** *Amer. J. Vet. Res., 49:* 1832-1835.

Report of the Scientific Committee for Animal Nutrition on the use of lasalocid sodium in feedingstuffs for turkeys. (Opinion expressed: 10 July 1991).

Terms of reference (March 1984):

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

1. Does the use of lasalocid sodium under the conditions proposed for feedingstuffs for turkeys (see Background) result in residues in animal products or excreted products which are qualitatively or quantitatively 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 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 Commission Directive 84/587/EEC(2), the use of lasalocid sodium is authorized at Community level under the conditions set out as follows in Annex I, Section D, of the Directive

(1) O.J. n° L 270, 14.12.1970, p. 1
(2) O.J. No. L 319, 8.12.1984, p. 13

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

The Scientific Committee for Animal Nutrition has expressed a favourable opinion on this use in its report of 14 December 1982

At present it is proposed to extend the authorization of use of this additive under the following conditions:

- Species or category of animals: Complete feedingstuffs for turkeys
- Maximum age: up to 16 weeks.
- Minimum and maximum content in complete feedingstuffs: 90-125 ppm (mg/kg).
- Other provisions: withdrawal period of at least three days before slaughter.

Opinion of the committee (July 1991)

Much of the basic information about lasalocid sodium has already been reviewed and evaluated in the Fourth Series of Reports of SCAN (see references). The proposal to extend the use to feedingstuffs for turkeys therefore requires only an evaluation of information specifically related to turkeys.

1. After administration for 8 weeks of 125 or 200 mg of lasalocid sodium per kg in feedingstuffs to groups of 5 or 6 turkeys as from one day, old residues of unchanged lasalocid were determined by HPLC/TLC (limit of detection 25 ppb) in muscle, liver, kidney, fat and skin after a 1, 2 and 3 day withdrawal period. Residues at 125 mg/kg dosage ranged from less than 0.025 to 0.085 mg/kg fresh tissue and at 200 mg/kg dosage from 0.025 to 2.25 mg/kg fresh tissue. No residues of unchanged lasalocid were detectable in any tissue after a 48 hour withdrawal period at 125 mg/kg, while for 200 mg/kg only skin contains detectable levels (0.038 mg/kg fresh tissue) after a 3 day withdrawal period.

The liver was identified as the target organ in terms of total residues when radiolabelled lasalocid was administered. After 5 days withdrawal total labelled residues were 0.85-0.89 mg/kg tissue lasalocid equivalents in the liver, 0.12 mg/kg in the abdominal fat and 0.07-0.11 mg/kg tissue in the kidney, skin and fat from non-abdominal sites.

These figures are very close to those reported for chickens. Unchanged lasalocid represents only 3.8% of the total residues in the liver, while extractable and non-extractable residues account for about 40% and 50% respectively.

Turkey excreta contained about 10% of lasalocid in unchanged form, the remainder representing a large number of different metabolites. This figure compares well with the 12% unchanged lasalocid present in chicken excreta. Chromatography confirmed a similar pattern of faecal metabolites for turkeys and chicken.

The metabolic fate of lasalocid in the turkey has been studied using ^{14}C-lasalocid labelled in three stable positions of the carbon skeleton. A metabolic balance determination established after the administration of daily doses (127 mg/kg feed) for 14 days that after 5 days of withdrawal from some 83.4% and 80.2% of the total administered dose had been were excreted in the droppings of the males and females respectively. Biliary excretion was considerable, indicating a significant absorption of the lasalocid.

The metabolic fate of lasalocid was compared in chickens and rats. Methodological difficulties arose because the molecule is metabolized into a very large number of metabolites, none of which accounts for more than 1% of the total radioactivity in the tissues or excreta. It may reasonably be concluded that lasalocid is metabolized similarly in the turkey and chicken but differently in the rat.

In view of the analytical difficulties in identifying the precise nature of the hepatic residues the Committee took into account exceptionally the very low bioavailability of these residues in addition to the above findings.

2. The Committee concluded in the light of the metabolic data that the residues of lasalocid in the liver of turkeys would not present a danger to the health of the consumer. As reported previously (see references) lasalocid has been found to be non phytotoxic and not to have herbicidal, insecticidal or insect growth regulatory activity. Concentrations of drug related material in the excreta are at least of an order of magnitude lower than the acutely toxic levels for aquatic species. There are no data on the end points of the breakdown of the molecule in the environment nor on the environmental effects of the metabolites of lasalocid present in turkey excreta. However the instability of lasalocid in aqueous systems at all pH values, particularly in the presence of light or heat, and in soil, particularly in the presence of chicken manure, permits the Committee to conclude that lasalocid related material in turkey excreta is unlikely to be prejudicial to the environment.

3. The Committee is of the opinion that the proposed conditions of use are acceptable i.e. a minimum and maximum content of lasalocid sodium of 90-125 mg/kg of complete feedingstuff for turkeys up to the age of 16 weeks but with a withdrawal period of at least 5 days before slaughter. This withdrawal period, which is similar to that recommended for chickens treated with lasalocid sodium, has been chosen because of the close comparability of the level and composition of the residues in the liver of the two species. Although the consumption of turkey liver from birds subject even to a zero withdrawal period would not lead to intakes exceeding the ADI of 0.005 mg/kg b.w., established on the basis of long-term studies in rats, the Committee nevertheless considered a withdrawal period of 5 days necessary because of the differences in metabolic handling of lasalocid by the rat and poultry.

REFERENCES:

- Dossiers submitted by Hoffmann La Roche in 1983 and 1990.

- Report of the Scientific Committee for Animal Nutrition on the use of Lasalocid Sodium in feedingstuffs for chickens. Fourth Series (1984). Report EUR 8769. Catalogue N° CD-NK-83-010-EN-C. Opinion expressed : 14 December 1982; p.106.

Report of the Scientific Committee for Animal Nutrition on the use of Salinomycin Sodium in feedingstuffs for pigs. (Opinion expressed: 10 July 1991).

Terms of reference (October 1986)

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

1. Has the use of the antibiotic salinomycin (sodium salt of polyether of monocarboxylic acid) at the dosages proposed for feedingstuffs for pigs (see Background) significant effects on the growth of the animal?

2. Is this use safe for the pig?

3. Can it result in the development of resistance in bacteria to prophylactic or therapeutic preparations or exert an effect on the persistence of Gram-negative bacteria in the digestive tract of the pig?

4. What is the metabolic fate of salinomycin in the pig? Does the proposed use result in residues in animal tissues? If so, what are the qualitative and quantitative composition and persistence of these residues?

5. Do the toxicological studies of the product allow to conclude that the proposed use does not present risks
   - for the consumer?
   - for the user?

6. Are the products derived from salinomycin and excreted by the pig of the same nature as those excreted by the chicken? If so, can these products be prejudicial to the environment?

7. 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 Commission Directive 84/587/EEC of 29 November 1984[2], the use of salinomycin is authorized at Community level under the following conditions set out in Annex I, Section D, of the Directive:

- Species of animal: chickens for fattening.
- Dosage: 50-70 mg/kg of complete feedingstuff.
- Other provisions: use prohibited at least five days before slaughter.

The use of salinomycin sodium in other animal species, which was also requested, is not authorized in the Community. In this connection, the Scientific Committee for Animal Nutrition expressed a provisional opinion in 1982 and, with regard to the chicken, a final opinion in 1984.

Additional files intended for supporting the application for the use of the product as growth promoter in pigs under the conditions mentioned below are presently available:

Use levels:

- piglets up to 4 months: 30-60 mg/kg of complete feedingstuff.
- pigs up to 6 months: 15-30 mg/kg of complete feedingstuff.

(1) O.J. No L 270, 14 Dec 1970 p.1.
(2) O.J. No L 319, 8 Dec 1984 p.13.

Opinion of the committee

Salinomycin is a polyether antibiotic of known structure. It is a monobasic carboxylic acid containing five cyclic ether rings.

1. Salinomycin has been used in a large number of trials both in the rearing of piglets and in fattening pigs in some 14 countries, including some in the EEC. The results are very variable and not easy to interpret because the pig populations involved were not homogeneous and the rearing conditions frequently not standardised. A consistent dose-effect relationship cannot be derived. However, a definite positive though rather flat response was discernible with increasing amounts of salinomycin in the feeds. This supports the existing practical experience of having to use more than the apparent lowest effective dose in view of the large inter-individual variations in biological response and the known differences in animal husbandry practice. A reasonable estimate of the doses giving a significant effect on growth would be 50 mg/kg feed (range 30-60) for piglets up to 4 months and 20 mg/kg feed (range 15-30) for pigs up to 6 months.

2. A six months study in pigs showed some hepatotoxicity at high doses, the NOEL being 2.5 mg/kg b.w. This is well above the dose proposed for growth promotion. Toxic signs following overdose in pigs, apparent after 5 days, are respiratory problems, anorexia, ataxia, inability to stand, hematuria and a mortality of about 10%. Histopathology indicates acute degenerative myopathy.

   In conjunction with administered tiamulin there is a dose-dependent increase in toxicity of salinomycin due to a reduction in excretion. The compatibility of tiamulin and salinomycin is influenced mainly by the total combined administered dose/unit time, estimated to be about 6 mg/kg b.w. There should also be an interval of at least 7 days between administration of tiamulin and salinomycin or vice versa, unless doses are of the order of 60 ppm salinomycin and 30-40 ppm tiamulin per pig. Attention is drawn to the possibility of adding a warning remark to the label of ionophores (salinomycin, monensin, narasin, maduramicin, lasalocid) stating that animals, including birds, should not be treated with products containing tiamulin while

receiving, and for at least 7 days before or after receiving, feed containing salinomycin because severe growth depression or death may result.

3. Salinomycin is moderately effective only against Gram-positive bacteria. No evidence for selection pressure or selection of indigenous enterococci with multiple-resistance plasmids was found. No cross resistance to 6 antibiotics used in human medicine was found and there is a lack of structural resemblance to chemotherapeutic agents used in human medicine. No resistance was observed in coccidia nor stable resistance by chromosomal mutations in *Staph. aureus In vitro*. Salinomycin alters to a small extent the relative levels of Gram-positive and Gram-negative bacteria in the intestine of pigs. There appears to be no need for concern over the possible development of bacterial resistance in the Gram-negative bacteria in the digestive tract of the pig.

4. 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. The amounts of radioactivity in rat and mouse tissues, particularly in the liver, were considerably larger than in chicken tissues.

   Salinomycin is rapidly metabolized in the gut and liver to numerous metabolites, mainly mono-, di- and trihydroxylated derivatives. The spectra of metabolites in rat and mouse liver were qualitatively and quantitatively similar, the spectrum in chicken liver being qualitatively similar but quantitatively different. Only 2-8% of liver radioactivity was non-extractable in the three species. Metabolism is slowest in the chicken. A similar study with ^{14}C-salinomycin in dogs indicated similar metabolites to those found in the chicken at about the same order of magnitude.

   Salinomycin is rapidly and extensively absorbed and metabolized by pigs when administered by the oral route. An average 83.5% of the radioactivity is excreted in the faeces with little intact

salinomycin being present, in urine around 2.1% is excreted. After 4 days, residues in the liver are 0.1mg/kg and less than the detection limit in muscle, kidney and fat (limit of detection of radiochemical method 0.01 mg/kg). Pig bile contains mainly the di- and trihydoxylated derivatives, while liver also contains other hydroxylated products, notably the monohydroxy derivative also found in rabbit liver.

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). After single dose administration to pigs 71-88% were found in the gastro-intestinal tract after 12 hours, radioactivity being only detectable in the liver. Repeated dose administration produced a liver residue level of 1.5 mg/kg after 8 hours withdrawal, falling to 0.4 mg/kg after 12 hours (monohydroxy-salinomycin absent, only di- and trihydroxy-salinomycin present), 0.2 mg/kg after 24 hours and 0.06 mg/kg after 60 hours, when analysed by a radiochemical method with a limit of detection of 0.01 mg/kg. When analysed by a microbiological assay with a limit of detection of 0.02 mg/kg no residues could be detected after 24 hours in the liver. The half-life of elimination was 2-3 hours during the first 12 hours after withdrawal and about 17 hours thereafter.

Pigs receiving repeated doses of salinomycin-containing mycelium in concentrations up to 200 mg/kg feed produced residues after 24 hours in the cutaneous fat of only 2 animals given 50 mg/kg feed. In another experiment no tissue residues were detected after 16 hours withdrawal. In the opinion of the Committee residues in the liver of pigs have fallen to an insignificant level after 24 hours withdrawal, the ionophoric activity being lower than residues in chicken liver. No residues are detectable in any other tissue of pigs after 12 hours withdrawal.

5. Most of the toxicity studies were performed with the dried mycelium and a few with 87-95% pure salinomycin. Acute toxicity studies in mice, rats, chicken, rabbits, dogs, pigs, bulls and horses showed oral LD_{50} values ranging from 21-60 mg/kg b.w.; for mice, rats, chickens and rabbits the signs of toxicity were mostly neurological.

Pigs, bulls and horses were increasingly sensitive in that order, toxic effects occurring mostly in the liver and myocardium. Acute dermal toxicity tests in the rat showed salinomycin to be moderately irritant. Salinomycin was non-antigenic and caused no immediate or delayed hypersensitivity in guinea-pigs. Subchronic studies were carried out in mice, rats, dogs and pigs. The target organs of toxicity were liver and spleen in mice (NOEL 3.75 mg/kg b.w.) and rats (NOEL 2.5 mg/kg b.w.), the nervous system in dogs (NOEL 1 mg/kg b.w.) and the liver in pigs (NOEL 2.5 mg/kg b.w.).

Two-year chronic toxicity studies were carried out in mice and rats and also a study over 2 1/2 years in rats using mycelium. Most of the observed adverse changes concerned organ weights and clinical biochemistry values but there was no evidence of carcinogenicity. The NOEL was <1.4 mg/kg in the mouse study and 2.5 mg/kg b.w. in the rat study.

A two-generation reproduction study in rats showed a NOEL of 5 mg/kg b.w. Embryotoxicity and teratogenicity studies in mice and rabbits revealed maternal and foetal toxicity in mice with a NOEL 4 mg/kg b.w. Rabbits showed increased resorptions most probably due to maternal/foetal toxicity with a NOEL of 0.25 mg/kg b.w. but no teratogenicity was observed.

The bioavailability of ^{14}C-salinomycin in pig liver was found in rats to be 10-31% of the administered dose. The ionophoric activity in pig liver was markedly lower than in chicken liver due to lower amounts of salinomycin. Genotoxicity was absent when salinomycin was tested in a bacterial system, a host-mediated assay in mice, a recessive lethal assay in *Drosophila* and a gene mutation test in mouse lymphoma cells. From the available toxicological studies an ADI of 0.003 mg/kg b.w. may be determined on the basis of the NOEL in the rabbit embryotoxicity study. It may be concluded from a consideration of the toxicity studies, the ADI of 0.003 mg/kg b.w. and the very low residues in pig liver that the proposed use of salinomycin does not present a risk to the consumer. As regards the user it should be noted that the substance is a dermal irritant.

6. 1.4-2.8% of the radioactivity of ^{14}C-labelled salinomycin fed to pigs are excreted in the urine and 81-86% appear in the faeces within 4 days, with small amounts of intact salinomycin present. Only about 1-5% of the microbiological activity in the feed is found in the excreta from broilers. Similar excretion patterns were found in other species (fowls, rats, mice, dogs). Because most of the metabolites have no antibiotic activity it appears likely that pig excreta, like chicken excreta, carry only very low antibiotic activity. There are no data on the toxicity of the faecal metabolites in pig manure.

There are no data on the stability of salinomycin in pig excreta during storage. Since salinomycin rapidly disappears from chicken manure, the concentration dropping from 0.04 ppm to 0.01 ppm within 6 days at room temperature, one can assume similar rates of disappearance from pig excreta.

The half-life of salinomycin in soil was 40-50 hours when measured microbiologically (limit of detection 0.01 mg/kg), only 1% remaining after 21 days, thus constituting practically no risk of accumulation. No data are available on leaching from soil or on the breakdown products in soil. Only very low concentrations of salinomycin are likely to be found in soil when using pig manure as fertilizer.

Salinomycin and its metabolites have a low toxicity for *Daphnia* (EC_{50} 24 h: 43 mg/l), no effect being observed at 21.5 mg/l. Fish toxicity (Golden orf-*Leuciscus idus*) was also low (LD_{50} 96 h: 30 mg/l). No effect was observed at 22.4 mg/l.

Total methane production from fresh pig manure from treated pigs was increased by 5% over that from untreated pigs. Salinomycin in the faeces of cattle reduced methane production by about 15%. At a salinomycin level of 8 mg/kg in soil (300 times the maximum concentration possible in soil under practical conditions) nitrification was slightly delayed. However no similar data are available for the effect of actual pig manure, i.e. of the metabolites, although most are devoid of antibiotic activity.

Plant growth was slightly inhibited at doses of 12.5-200 mg salinomycin/m^2 for carrots, kohlrabi, chinese cabbage, potatoes and sugar beet. No uptake into plants was noted at 14 mg/kg soil. These tests were done with pure salinomycin and not with manure containing metabolites. These observations indicate that possible harm to the environment is unlikely at the concentrations reached by normal use of pig manure. However, data on the effect of the metabolites are lacking.

7. In the light of the available evidence the Committee is of the opinion that the use of salinomycin in the feedingstuffs of pigs at the proposed levels could be admitted. A similar warning remark regarding interaction with tiamulin should be included on the label of ionophore treated feed and tiamulin preparations.

References

- Dossiers submitted by Hoechst in 1984 and 1987

- Report of the Scientific Committee for Animal Nutrition on the use of salinomycin in feedingstuffs (Chickens, piglets, pigs and cattle. Provisional pending on reassessment). Fourth Series (1984). Report EUR 8769. Catalogue N° CD-NK-83-010-EN-C. Opinion expressed : 14 April 1982; p. 36.

- Bährecke, G., Baginski, M., Dost, G. and Rudolf, B.S. 1985. **Dosis-Wirkungsbeziehungen von Salocin^R in der Aufzucht und Mast von Schweinen.** *Kraftfutter, 4*: 130-134.
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Report of the Scientific Committee for Animal Nutrition on the use of Narasin + Nicarbazin in feedingstuffs for chickens. (Provisional opinion : 10 July 1991).

Terms of Reference (June 1990)

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

1. Has the use as a coccidiostat of **Narasin** (polyether of monocarboxylic acid produced by *Streptomyces aureofaciens*) plus **Nicarbazin** (equimolecular complex of 1,3-bis (4-nitrophenyl)urea and 4,6-dimethylpyrimidine-2-ol) under the conditions proposed for its use as an additive for fattening chickens (see background) significant effects on the prevention of coccidiosis in this animal species?

2. Is this use safe for the chickens?

3. Can it be monitored in animal feedingstuffs?

4. Can it result in the development in bacteria of resistance to prophylactic or therapeutic preparations?

5. What is the metabolic fate of the whole product Narasin+Nicarbazin in the chicken? Are the two component products compatible? Does the proposed use result in residues in animal tissues? If so, what are the qualitative and quantitative composition and persistence of these residues?

6. Do the toxicological studies allow to conclude that the proposed use does not present risks
   - for the consumer?
   - for the user?

7. What are the nature and the persistence of the excreted products derived from Narasin+Nicarbazin? Can these products be prejudicial to the environment?

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

Background

Narasin(1) and Nicarbazin(2) are coccidiostats already included in section D (Coccidiostats and other medicinal substances) of the Annex I list to Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs(3).

The preparation Narasin+Nicarbazin was the subject of an application for admission in the same section D (Coccidiostats and other medicinal substances) of this Council Directive 70/524/EEC (3) under the following conditions of use:

- Species of animal: chickens for fattening.
- Use level: 40+40 to 50+50 mg/kg of complete feedingstuffs.
- Other provisions: Use prohibited at least five days before slaughter.

Opinion of the Committee

1. Narasin is a polyether antibiotic produced by deep culture fermentation of a strain of *Streptomyces aureofaciens*. Its chemical structure shows it to be a monobasic carboxylic acid containing 5 cyclic ether rings.

(1) J.O. No. L183, p.15 (11.07.1984)
(2) J.O. No. L310, p.19 (5.11.1986)
(3) J.O. No. L270, p. 1 (14.12.1970)

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-dimethyl-pyrimidine (HDP).

In a large number of tests, using various combinations ranging from 10+10 to 60+60 mg/kg of feedingstuff of each substance against 5 *Eimeria* strains producing intestinal coccidiosis and 1 strain producing caecal coccidiosis as well as mixed *Eimeria* infections, a combination of 50+50 mg/kg of complete feedingstuff has been the most efficient with regard to growth, improved feed efficiency, lesion control and oocyst elimination. A clear dose-response relationship has been demonstrated. Efficiency of the combination has been compared with feedingstuff containing 70 mg/kg Narasin or 125 mg/kg Nicarbazin but not with other coccidiostats. Because the 50+50 combination does not eliminate oocysts completely, sufficient remain to induce immunity. The 50+50 combination is effective against resistant, strongly pathogenic single strain infections and mixed infections. However, with this dosage there are the occasional cases where weight gain is reduced and feed efficiency impaired.

2. The use of the 50+50 mg/kg of complete feedingstuff combination is safe for chicken. The occasionally observed smaller weight gain or impaired feed efficiency is acceptable in the absence of any other adverse effects in chicken, when compared to the risk of 30 - 35% mortality from untreated infection.

3. There are adequate analytical methods for assaying the pure drugs and the tissue residues. Narasin is assayed by TLC with bioautographic overlay using *Streptococcus faecium* (limit of detection 0.005 mg/kg). Nicarbazin is assayed mostly by determining the DNC component with HPLC/UV (limit of detection 0.10 mg/kg), less frequently by HDP determination using FID GLC (limit of detection 2 mg/kg).

4. Of the two components only Narasin has antibiotic activity. The evaluation by SCAN is reported in the Fourth Series of Reports. SCAN concluded that none of the gram-positive bacteria, the only kind against which Narasin is active, showed cross-resistance to a variety of clinical

antibiotics tested, even when transient resistance to Narasin developed. There appears therefore to be no need for concern over the possible development of bacterial resistance. A separate experiment on the possible effect of Nicarbazin on the antibacterial activity of Narasin against *E. coli*, *Bacteroides* and *Staphylococci* was negative, showing no change in MIC for the susceptible and non susceptible strains examined, however only little material was used. The normally susceptible *Staphylococci* were found to be unexpectedly resistant to the action of narasin.

No explanation was offered for this observation. It would therefore be desirable to repeat the experiment or to provide an explanation for the change in the MIC values. Because preparations effective against gram-positive bacteria frequently extend Salmonella shedding through an imbalance in the intestinal flora, it could be desirable to determine the effect of the combination on Salmonella shedding. In a separate experiment Nicarbazin was shown to have no antibacterial effect on a selection of bacterial strains.

5. Nicarbazin is largely absorbed by the chicken, HDP is excreted mainly in the urine and DNC mainly in the bile and faeces. HDP is rapidly metabolized and disappears from all tissues after a 5-day withdrawal period (limit of detection 0.3-0.4 mg/kg tissue). No identification of metabolites is available. DNC is metabolized slowly with residues persisting mainly in the liver, amounting to 0.06-0.08 mg/kg tissue DNC equivalents after 11 days withdrawal. HDP therefore contributes only 4.5% of the total Nicarbazin residues. Use of ^{14}C-labelled DNC in the chicken indicates the existence of 2 main metabolites which are acetylated amines arising from the reduction of one or both nitro groups. A third minor metabolite results from cleavage of the carbanilide group followed by reduction and acetylation of the nitro group. Excreta consist mainly of DNC plus small amounts of the 3 metabolites. Liver residues consist mainly of about 80 % DNC, less than 12% metabolites (M1 + M3) and some 3.3% unidentified matter. Metabolism in the rat is similar to that in the chicken.

Narasin is well absorbed and largely excreted in the bile. Excreta contain 6 major metabolites which incorporate the dihydroxy and

trihydroxynarasin structure. In total they account for only 20% of the parent antibiotic activity and for a very weak ionophoric activity. The metabolism in the rat is similar. At zero withdrawal time there is no measurable radioactivity in muscle and a rapid decline in other tissues. After three days the liver and skin contain 0.04 and 0.025 mg/kg Narasin equivalents respectively. 5% of the total liver radioactivity and 50% of the total radioactivity in fat at zero withdrawal time was Narasin. None could be detected after 3 days withdrawal using a microbiological assay (sensitivity 0.005 mg/kg).

Narasin does not change the metabolic pathway of DNC although the metabolic rate seems to be changed. Residues are higher by 25, 50, 38, 41 and 35% in liver, muscle, kidney, fat and skin respectively which implies an effect on metabolic flux rates. 90 % of DNC disappears after 3 days and 98% after 5 days in all tissues. Residues after 5 days are less than 0.025 mg/kg DNC equivalents in muscle, fat and skin, 0,22 mg/kg in liver and 0.14 mg/kg in kidney. After 7 days liver residues amount to 0.06 mg/kg, kidney residues to 0.03 mg/kg. Unchanged DNC is the major residue (over 80%) in fat and skin but less in muscle as determined by HPLC (limit of detection 0.05 mg/kg). 60% of liver radioactivity at zero withdrawal time and 45% after 5 days are DNC. The corresponding kidney residues are 25% and 12% respectively.

No data are available on the effect of Narasin on HDP residues. Although no direct data on the effect of Nicarbazin on Narasin metabolism are available, there is evidence that the residue pattern of Narasin is not modified.

In chickens, raised with 125 mg/kg feed of Nicarbazin, a steady state residue level of 240-390 µg/kg liver and 8-10 µg/kg muscle is still found after 3-4 weeks withdrawal due to cross contamination from stable Nicarbazin residues in the litter. Despite the low tissue residues and the absence of significant differences there is a trend to increasing residue levels with time. Moreover the use of an average of 4 chickens as the experimental unit per time period makes statistical interpretation difficult. Comments are therefore required on this situation.

6. Narasin and nicarbazin have been individually assessed for their toxicity in the chicken, the rat and the mouse. These data are summarized in the Fourth Series of Reports of SCAN. The ADI then established for Narasin was 0.0038 mg/kg b.w. and for Nicarbazin the ADI was 0.20-0.24 mg/kg b.w. The ADI applies to the mixture of DNC + HDP. Separate ADIs were not established for DNC or HDP. Studies in chickens with various combinations showed that the NOEL was 62.5 + 62.5 mg/kg feed. Studies in laboratory animals involved acute toxicity, dermal and ocular toxicity but hypersensitivity and inhalation toxicity with the granular material were not tested. A 90-day study in rats, using various combinations, showed no treatment-related effects except for a slight reduction in body weight for the 60+60 combination.

   Neither Narasin nor Nicarbazin have shown any carcinogenic potential in long-term studies. Extensive mutagenicity studies have shown no evidence of genotoxicity. Since the tissue residues were not qualitatively different from those arising from use of the single compounds, long-term studies o n the combination are not considered necessary.

   A teratogenicity study in rats showed no teratogenic potential, the no-effect-level with respect to maternal and foetal toxicity being 0.75+0.75 mg/kg b.w.

Bioavailability was not studied because of the very low residues.

   The Committee considered that the use of the combination did not present any toxicological risk for the consumer or the user.

7. The only data available on the substance and metabolite levels in chicken excreta concern the two constituents separately. Excreta contain primarily parent DNC and small amounts of 3 metabolites. At the

recommended dosage of 50 mg/kg feed Nicarbazin about 40 mg/kg of drug related material in the excreta could be estimated, of which about 28 mg/kg excreta is DNC equivalents and 12 mg/kg HDP equivalents. Almost 99% of Narasin appears in the excreta, hence a 50 mg/kg feed dosage would result in 46 mg/kg excreta of which 30% or 14 mg/kg excreta is parent narasin, while 20% or 9 mg/kg excreta are hydroxylated metabolites. The fate of the remaining 50% is not known. The antibiotic activity of the excreted products is however low. There are no data on the biodegradation of Nicarbazin or its metabolites in chicken manure. Narasin does not degrade in chicken manure to any significant extent even after 18 months storage.

The DNC component of Nicarbazin is very stable in soil with a half-life of about 49 weeks. The degradation products have not been determined. There is no leaching from treated soil as demonstrated by radioactivity measurements and absence of formation of $^{14}CO_2$. The behaviour of the water soluble HDP is not documented nor is there any information available on the degradation products of HDP.

Narasin degrades rapidly in soil, some 92% within 4 weeks, particularly in the presence of Nicarbazin. A similar kinetic profile is obtained with chicken litter containing Narasin. The degradation products of Narasin are unknown. Basic soils leach more Narasin but, as it is practically insoluble in water, there is unlikely to be any significant passage into surface waters.

The acute NOEL for the combination is 0.5 mg/l for *Daphnia magna*, the 48 h EC_{50} is 20.65 mg/l. The acute NOEL is 1.80 mg/l for the Bluegill and 0.16 mg/l for the Rainbow trout.

No drug-related phytotoxicity is noted when seeds of corn, soybean, wheat and tomato are fertilized with chicken excreta from birds fed the 50+50 combination. Levels over 16 mg/kg soil reduce growth and cause sublethal toxicity after 7 days in *Lumbricus terrestris*. Toxicity is almost entirely attributable to Narasin. Metabolites have not been specifically investigated.

Nitrification is not affected and no effects on methanogenesis are observed.

The Committee concluded that in view of the relatively low concentration of parent drugs and metabolites in chicken excreta, the low solubility of Narasin and the DNC component of Nicarbazin in water, the indirect evidence of lack of bioaccumulation of Narasin, and the absence of data on significant environmental toxicity, no adverse effects on the environment would be expected under ordinary conditions of use. Data on the fate of HDP and the unidentified metabolites of Narasin are desirable.

8. On the basis of the above data the Committee is of the opinion that the use of Narasin+Nicarbazin can be admitted without risks in the feedingstuff for chickens at the levels provisionally authorized of 50+50 mg/kg feedingstuff and with a withdrawal period of at least 5 days before slaughter. It would be desirable to obtain information on: the metabolic fate of HDP; on the effect of Narasin on HDP residues; on the unidentified metabolites of Narasin, on the biodegradation products of Narasin, Nicarbazin and their metabolites in chicken manure and the soil; on the fate of HDP in the soil; and on the effect of administration of the combination on Salmonella shedding.

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- Second report of the Scientific Committee for Animal Nutrition on the use of narazin in feedingstuffs for chickens. Fifth Series (1986). Report EUR 1041EN. Catalogue N° CD-NK-86-003-EN-C. Opinion expressed : 8th February 1984; p.4.

- Second report of the Scientific Committee for Animal Nutrition on the use of Nicarbazin in feedingstuffs for fattening chickens. Fifth Series (1986). Report EUR 1041EN. Catalogue N° CD-NK-86-003-EN-C. Opinion expressed : 5 July 1985; p.33.

Report of the Scientific Committee for Animal Nutrition on the use of Diclazuril in feedingstuffs for Chickens. (Opinion expressed: 10 July 1991)

Terms of reference (June 1990)

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

1 Has the use as a coccidiostat of Diclazuril (2,6-dicloro-alpha-(4-chlorophenyl)-4-(4,5 dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl) benzeneacetonitrile) under the conditions proposed for its use as an additive for fattening chickens (See Background) significant effects on the prevention of coccidiosis in this animal species?

2 Is its use safe for the chicken?

3 Can it be monitored in animal feedingstuffs?

4 Can it result in development of resistance in bacteria to prophylactic or therapeutic preparations?

5 What is the metabolic fate of Diclazuril in the chicken? Does the proposed use result in residues in animal tissues? If so, what are the qualitative and quantitative compositions and persistence of these residues?

6 Do the toxicology studies allow to conclude that the proposed use does not present risks:
- for the consumer?
- for the user?

7 What are the nature and the persistence of the excreted products derived from Diclazuril? Can these products be prejudicial to the environment?

8 In the light of the answer to the above questions, are the proposed conditions of use acceptable?

Background

Diclazuril was the subject of an application for admission in Section D (Coccidiostats and other medicinal substances) of Council Directive 70/524 EEC(1) of 23 November 1970 concerning additives in feedingstuffs under the following conditions of use:
- Species of animal: chicken for fattening.
- Use level: 1 ppm in the feed.
- Other provisions: use prohibited at least three days before slaughter.

Opinion of the Committee

Diclazuril is a slightly yellowish to beige powder with a melting point at 292-297 °C and with a maximum impurity level (by HPLC) of about 1.5%. Chemically Diclazuril is a 2,6-chloro-alpha-(4-chlorophenyl)-4-(4,5-dihydro-3,5- dioxo-1,2,3-triazin-2(3H)-yl) benzeneacetonitrile, a compound practically insoluble in water and moderately soluble in N-dimethylformamide (3.26%) and DMSO (4.8%). Its use in feedingstuffs, as a premix prepared by adding 5 g to 995 g of partially deproteinized soya flour, is recommended at a final level of 1 ppm.

1 From preliminary cage-trials performed, in EEC and extra-community countries, by administering the additive (from 0.5 up to 10 ppm in

(1) J.O. No. L270 p.1 (14.12.1970)

feed) to chickens infected by a single *Eimeria* strain or mixed *Eimeria* strains, Diclazuril showed anticoccidial activity at all the doses tested.

Floor pen trials performed in Belgium, Germany, South Africa, Yugoslavia, Spain, Italy, United Kingdom, USA and Denmark on some thousand animals revealed a complete anticoccidial activity of the active principle at the dose level of 1 ppm in feedingstuffs.

The same finding emerged from field trials on spontaneously infected chickens in which the proposed level of 1 ppm in feedingstuffs consistently improved the intestinal lesion scores and the productive performance of treated animals.

The exact mode of action of Diclazuril is not known but it has a potent coccidicidal action on some E. strains (*E. tenella and E. acervulina*) and coccidiostatic action on others.

2 Administration of Diclazuril, at the proposed level in feedingstuffs (1 mg/kg), to chickens proved to be safe for these animals. When broiler chickens were fed for 37 days from birth with a diet fortified with 25 ppm of the drug (i.e. 25 times the recommended dose) they showed no signs of intolerance. This finding indicates a very large safety margin for Diclazuril in the target species. Identical conclusions were drawn from experiments performed on female and male breeding broilers, fed with a diet at 5 ppm of Diclazuril for 28 and 42 days respectively, as well as on ducks and turkeys, receiving for 7 days diets fortified with Diclazuril at the level of 10 and 100 ppm respectively.

Comparative tolerance experiments, carried out in mammals, showed that rabbits fed with a 20 ppm Diclazuril diet for 16 days did not show any sign of distress or malaise, horses well tolerated a daily oral dose of 1 mg/kg b.w. for 6 consecutive days and dogs and cattle an oral dose as high as 10 mg/kg b.w.

3 The active principle may be monitored in animal feedingstuffs by means of a GC-method (detection limit = 30 ppb) or of a HPLC-method (detection limit = 100 ppb). An HPLC-method has also been described which allows the detection of the active principle in biological samples from treated animals at the sensitivity level of 50 ppb (plasma, muscle, liver and kidney) and of 100 ppb (skin and fat).

4 No evidence of possible bacterial resistance has been reported. Since the additive is practically devoid of antibacterial as well as antimycotic properties, up to a concentration of 100 µg/ml, it is unlikely that the use of Diclazuril willresult in development of resistance to prophylactic or therapeutic preparations.

No resistance to Diclazuril has been observed in any of 119 *Eimeria* strains collected from naturally infected pens. Though data on the long-term use of Diclazuril in the field are not available, some experiments *in vitro* have shown occasional coccidial resistance developing after 13 contact-passages in media containing 0.1 ppm of the additive, thus based on available data, the induction of resistance is a slow occurring phenomenon.

5 Diclazuril is excreted at a relatively slow rate (50% after 24 hours, 85% and 95% after 5 and 10 days respectively), mainly through the faeces. It is metabolized to a limited extent, the additive being mainly excreted unchanged. A major metabolite accounting for 5.6-8.3% of the administered administered was detected in the excreta as well as nine other compounds all accounting for less than 2% in total. As none of these metabolites were found in the plasma and tissues, it was hypothesized that they are produced by the intestinal flora. The main metabolite was tentatively identified as 4-amino-2,6-dichloro-alpha-(4-chlorophenyl) benzeneacetonitrile resulting from the opening of the triazine ring.

In the rat Diclazuril showed very poor absorption and a metabolic pattern differed from that observed in the chicken. Following repeated ^{14}C-labelled Diclazuril administration at 1 ppm level,

kinetic analyses showed the same monoexponential decline ($t_{1/2}$ ≈ 50-60 hours) of the total radioactivity in the plasma and tissues during the withdrawal period. Liver proved to be the target tissue. The total residues, expressed as unchanged Diclazuril, were 386 ng/g after 6 hours and 240, 187 and 107 ng/g (ppb) after 24, 72 and 120 hours respectively.

Of these residues unchanged Diclazuril represented 95.8, 84.2, 74.5 and 79.9% respectively, the metabolites identified in the excreta did not account for the remaining radioactivity. After a 3 day withdrawal period residues were below the practical detection limit of Diclazuril in the muscle, the skin and the fat. These results were confirmed from a study carried out under field conditions using unlabelled Diclazuril at the proposed dosage.

The MRLs calculated according to different withdrawal times from 0 to 10 days lie consistently under the calculated ADI, i.e. 0.022 mg/kg b.w. which corresponds to 1.32 mg for a 60 kg human consumer.

6 The data on acute (oral LD_{50} higher than 500 mg/kg b.w. in mice, rats, dogs and chickens) as well as chronic toxicity (NOEL at 2.2 and 3.15 mg/kg b.w./day oral administered for 24 months in mice and 30 months in rats respectively) indicate low general toxicity. The NOEL in the mouse study has been used for establishing the ADI of 0.022mg/kg b.w.. Diclazuril is devoid of eye irritating properties (rabbit) or sensitizing potential (guinea-pig), nor is it mutagenic, teratogenic or carcinogenic in animal tests. Taking into account these data and those emerging from the metabolic studies, the Committee is of the opinion that the proposed use of Diclazuril should not result in risks either for the consumer or the user.

7 Because of the high stability of Diclazuril, a major concern for the Committee has been that Diclazuril's slow degradation rate might result in some accumulation in the environment. Thus Diclazuril in different soil types degrades with a half-life longer than 74 days and that its degradation is only slightly enhanced when in manure.

Studies on the potential of Diclazuril for environmental effects have indicated however that they may be predicted to be insignificant because of its low toxicity and low inclusion level in feed.

Based on the concentration found in the excreta (280 to 710 µg/kg) and a manuring regimen of 10 tons/ha/year on the same pasture without any degradation or migration of the residues, this could yield an accumulated burden of Diclazuril up to 2.8 - 7.1 µg/kg (ppb) soil *per annum*, i.e. a pollution level greatly below not only the toxic, but also the effective concentration of the compound in the complete feed. In view of this observation, although some accumulation in the environment of this persistent compound may occur, it is anticipated not to result in ecotoxicological hazards.

8 The conditions of use of Diclazuril proposed by the Commission, i.e. a provision of a 3-day withdrawal period can be accepted by the Committee since this withdrawal period includes a high margin of safety.

References

- Dossier submitted by Janssen Pharmaceutica

Report of the Scientific Committee for Animal Nutrition on the use of Propane-1,2-diol (Propylene glycol; 1,2-Propanediol), as a feed additive in the feedstuffs for cats. Provisional opinion expressed: 15 January 1991.

Terms of reference (November 1990)

1. May Propane-1,2-diol (E-490), at the level permitted in the feeding-stuffs (see background), adversely affect the health of the cats?

2 Is the level permitted in feeding-stuffs such that an usage distinct from that of heading G ("preservatives") of Council Directive 70/524/EEC is excluded?

3. Can the maximum level be reduced from its permitted level (see background) without affecting the desired "preservative" properties in feedingstuffs for cats?

4. Can other additives listed under the same heading G ("preservatives") satisfactorily replace Propane-1,2-diol?

Background

Additive E-490 is included in Annex I of Directive 85/520/EEC of 11 November 1985 amending the Annexes to Council Directive 70/524/EEC concerning additives in feeding-stuffs[1] under heading G

(1) O.J. No. L319, 08.12.84,p.13.

(Preservatives) at a maximum level of 75 000 mg/kg (equivalent to 7.5 % w/w fresh matter basis). It is claimed that when the additive is fed to cats over a prolonged period of time, erythrocytes may develop oxidative damage to the haemoglobin, Heinz body formation and, possibly, reduced resistance to oxidative stress. Therefore the Commission Services would like to know if the permitted amount will lead in practice to significant higher levels when expressed per unit dry matter basis, and whether it presents a danger to the wellbeing of cats.

The Government of the Federal Republic of Germany, wrote to the Commission in the following terms:

In the Annex to this letter, a report by Hickman et al. shows an increased presence of Heinz body formation within erythrocytes in kittens fed on a diet of a feedingstuffs containing Propane-1,2-diol. From the results of the experimental survey , it is manifestly clear that Propane-1,2-diol increases the occurrence of Heinz bodies. This was already known from the data presented before authorising the product and it was also clear it could be caused by other factors in this species of animal. It was not, however, known that relevant physiological parameters are changed. However, in the survey by Hickman et al., it has been proven that feeding with Propane-1,2-diol significantly shortens the life-span of erythrocytes. This state of affairs sheds new light on the question of absence of danger to health in feedingstuffs for cats containing Propane-1,2-diol because, as indicated in the survey, the shortened life-span of the erythrocytes could, when the animals are specifically stressed (illnesses, intoxication) have negative consequences on animal health. The Government of the Federal Republic of Germany is therefore urging the Commission to put the use of Propane-1,2-diol for cats on the agenda of the next meeting of the Committee of Experts on Additives which will be held on 19 September 1990. On this occasion, a decision needs to be taken to suspend the use of the aforesaid additive until the damaging suspicions have been eliminated through further experimentation.

Opinion of the Committee

The SCAN considered the communication received from the Federal Republic of Germany on the production of Heinz bodies by large doses of 1,2-Propanediol in the food for cats and considered, provisionally that there was no immediate need to suspend the use of this substance.

New references were distributed among members of the Toxicology group. The experts also examined a dossier that has not been examined before by SCAN presented by FEDIAF in 1983(2). On the basis of which admission was granted by Commission Directive 87/552/EEC of 17 November 1987(3) amending the Annexes to Council Directive 70/524/EEC concerning additives in feedingstuffs(4) . This dossier addressed the issues of whether Heinz body formation should be interpreted as a significant adverse effect in the cat, and justification for the level of inclusion.

The SCAN experts agreed that this information should be examined, together with that provided by the Federal Republic of Germany before seeking to answer the questions mentioned in Annex VII and before seeking further data from industry. In the meantime, the declaration made at the 71 meeting (see below), is still valid.

"Before coming to any decision on the continued use of this substance in pet-food the Committee required the provision of adequate data on the toxicology of 1,2-Propanediol. SCAN did not consider that there was an immediate need to suspend the use of this substance before it had an opportunity to assess its safety-in-use as an ingredient of pet-food."

(2) Fédération Europeenne de l'Industrie des Aliments pour Animaux Familiers (F.E.D.I.A.F.) 1983. "1,2-Propylene Glycol (Propylene Glycol) in Soft-Moist Cat Foods, Part 1 (250 pp) and Part II (Appendix I to XI, 293 pp). Brussels (Belgium): Square Marie-Louise, 18.

(3) OJ No L 336, 26.11.87, p. 34

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

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- Hapke H.J. & Majid H. (1983). **"Hamolytische Wirkungen von Losungsmitteln, 2. Effekte ausgewahlter organischer Losungsmittel"** *Deuts. Tierarztl. Wochenschr.*, **90**:216-218.

GUIDELINES FOR THE ASSESSMENT OF ADDITIVES IN FEEDINGSTUFFS

Amendments proposed to modify the Guidelines for the Assessment of Additives in Animal Nutrition [Council Directive 87/153/EEC of 7 March 1987(1)]. to make this document also applicable for microorganisms and/or enzyme preparations intended for nutritional purposes

Provisional Opinion Expressed July 10 1992.

GENERAL ASPECTS

These guidelines are intended as a guide for establishing dossiers on substances and preparations being submitted for authorization as additives in feedingstuffs. These dossiers must enable an assessment to be made of the additives based on the present state of knowledge and make it possible to ensure their compliance with the fundamental principles laid down for their admission, which are the subject of the provisions of Article 7 (2) of Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs(2).

All the studies outlined in these guidelines may be required and, if necessary, additional information will be requested. As a general rule, studies to establish the identity, conditions of use, physico-chemical properties, methods of determination and efficacy of the additive, and also its metabolism, biological and toxicological effects on target species must be provided. The studies necessary for the evaluation of risks to human health or the environment will depend essentially on the nature of the additive and the circumstances of its use. In this

(1) Spec. No. 4 (87) p.69 is applicable.
(2) O.J. No. L270 (14.12.70) p.1, and O.J. No. L319 (08.12.84) p.13

It may not always be necessary to subject additives intended exclusively for pet food to be as exhaustive a programme of chronic toxicity, mutagenicity and carcinogenicity testing as that required for additives intended for feeding to livestock from which product for human consumption is derived. To determine chronic toxicity, studies on two target species or on one target species and rats for a period of one year will generally suffice. Mutagenesis and carcinogenesis studies can generally be dispensed with if the chemical composition, practical experience, or other considerations do not indicate the likelihood of changes. It is possible to dispense with the analysis of residues in pet animals.

Knowledge of the metabolism of the additive in food producing stock, of the residues and their bioavailability is essential. In particular it must enable the extent of the toxicological studies to be performed on laboratory animals in order to assess the risks, if any, to the consumer to be determined. This evaluation cannot be based solely on data confined to determining the direct effects of the additives on laboratory animals. The latter do not provide specific information on the actual effects of residues resulting from the metabolism in the species for which the additive is intended.

Any application for authorization of an additive or a new usage for an additive shall be supported by a dossier which should include detailed reports presented in the order and with the numbering proposed in these guidelines. Reasons must be given for the omission from the dossier of any data prescribed in these guidelines. Publications to which reference is made must be attached to it. The reports of experiments must include the plan and reference number of the experiment, detailed description of the tests, results and their analysis, and also the name, address and signature of the person responsible for the study. A statement from the person responsible for Good Laboratory Practice regarding observance of such practice is to be attached to the reports.

The determination of physico-chemical, toxicological and ecotoxicological properties shall be performed in accordance with the methods established

by Commission Directive 84/449/EEC of 25 April 1984 adapting to technical progress for the sixth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances[3], or with methods internationally recognized by scientific bodies. The use of other methods should be justified.

Each dossier shall contain an adequate summary. The dossiers relating to antibiotics, coccidiostats and other medicinal substances, growth promoters micro-organisms and/or enzyme preparations, must be accompanied by a monograph, conforming with the model provided in Section V, enabling the additive concerned to be identified and characterized in accordance with Article 8 (1) of Directive 70/524/EEC.

The term 'additive', as used in these guidelines, refers to the active substances or the preparations containing active substances in the state in which they will be incorporated in premixtures and feedingstuffs.

The Commission must be notified within a reasonable time by the Member State which forwarded the dossier to it of any modification to the manufacturing process or the composition of an additive, its field of application or its conditions of use. This could necessitate the submission of documentation suitable for a new assessment. These requirements will be especially necessary for products derived from micro-organisms, the genetic characteristics of which have been modified or which arise as natural mutants.

(3) O.J. No. L251 (10.09.84) p.1.

CONTENTS

SECTION I

SUMMARY OF THE DATA IN THE DOSSIER

SECTION II

1. Identity of the additive

1.1 Proposed proprietary name(s)

1.2 Type of additive according to its main function

1.3 Qualitative and quantitative composition (active substance, other components, impurities)

1.4 Physical state, particle size

1.5 Manufacturing process including any specific processing procedures

N.B. If the active substance is a mixture of active components, each clearly definable, main components must be described separately and the proportions of the mixture given.

2. Specifications concerning the active substance

2.1 Generic name, chemical name according to IUPAC nomenclature, other non-proprietary and generic international names and abbreviations. Chemical Abstracts Service Number (CAS).

If the active substance is a micro-organism: name and taxonomic description according to the international Codes of Nomenclature. Other internationally recognized Manuals of Systematics can also be used(5).

For enzyme preparations : name according to main enzymic activities as described by IUB/IUPAC, EINECS and CAS Number.

As a general principle micro-organisms and/or enzyme preparations should be derived from non-pathogenic and non-toxicogenic microbial sources.

(5) Such as Bergey's Manual of Determinative Bacteriology. "The Yeasts, a taxonomic study" by Lodder, "A Dictionary of Fungi" by Ainsworth and Bisley or "The Genus Aspergillus" by Raper and Fennel.

2.2 Formula, empirical and structural, and molecular weight. Qualitative and quantitative composition of the main components, if the active substance is a fermentation product.

For micro-organisms : name and place of culture collection, if possible one in an EEC collection, where the strain is deposited and depositing number, genetic modification and all relevant properties for its identification. In addition, origin, appropriate morphological and physiological characteristics, developmental stages, relevant factors that may be involved in its biological activity (as an additive), resistance pattern, DNA-DNA homology data and plasmid profile. Number of colony forming units (CFU) for each species .

For enzyme preparations : the biological origin, the activities towards relevant chemically pure model sustrates and other physico-chemical characteristics.

2.3. Degree of purity

For micro-organisms : genetic stability and purity of strains cultivated.
For enzyme preparations : purity (checking the level of contaminating micro-organisms, heavy metals, absence of mycotoxins and antibacterial activity),and composition of the non-enzymic component derived from the source material.

Qualitative and quantitative composition of the impurities.

2.4. Relevant properties

For chemicals: Electrostatic properties, melting point, boiling point, decomposition temperature, density, vapour pressure, solubility in water and organic solvents, mass and absorption spectra and any other appropriate physical properties.

For micro-organisms : relevant biological properties.
For enzyme preparations: optimal pH, temperature and other appropriate properties.

2.5. Manufacturing, purification processes and media used. Variation in the composition of the batches in the course of production.

3. Physico-chemical, technological and biological properties of the additive.

3.1. Stability (for micro-organisms loss of biological activity e.g. viability) on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Expiry date.

3.2. Stability (for micro-organisms loss of biological activity, e.g. viability) during the preparation of premixtures and feedingstuffs, in particular stability to heat, pressure and moisture. Possible decomposition products.

3.3. Stability (for micro-organisms loss of biological activity, e.g. viability) during the storage of premixtures and feedingstuffs (storage time under defined conditions). Expiry date.

3.4. Other appropriate physico-chemical, technological or biological properties such as ability to obtain homogeneous mixtures in premixtures and feedingstuffs, dust-forming properties, and for micro-organisms and/or enzyme preparations, assessment of resistance to degradation or loss of biological activity in the digestive tract or by systems of simulation *in vitro*.

3.5. Physico-chemical or biological incompatibilities or interactions (e.g. with feedingstuffs, other additives or with medicinal products).

4. Conditions of use of the additive.

4.1. Proposed use in animal nutrition (e.g. species and category of animal, type of feedingstuff, period of administration and withdrawal period).

4.2. Contra-indications.

4.3. Proposed dosing in premixture and feedingstuffs (expressed as a percentage of the active substance by weight or appropriate units of biological activity such as CFU/per gram of product for micro-organisms or relevant activity units for enzyme preparations, for premixtures; and in mg/kg for feedingstuffs).

4.4. Other known uses of the active substance or the preparation (e.g. in foodstuffs, human or veterinary medicine, agriculture and industry). For each use give the proprietary names, indications and contra-indications.

4.5. If necessary, measures for the prevention of risks and means of protection during manufacture and handling.

5. Control methods.

5.1. Description of the methods used for the determination of the criteria listed under items 1.3., 2.3., 2.4., 2.5., 3.1., 3.2., 3.3., 3.4. and 4.3.

5.2. Description of the qualitative and quantitative analytical methods for routine control of the additive in premixtures and feedingstuffs.

5.3. Description of the qualitative and quantitative analytical methods for determining residues of additives in animal produce.

N.B. The methods specified and the results should be accompanied by information as to percentage recovery, specificity, limits of detection, possible interferences, reproducibility and to the sampling method used. Reference standards of the preparation and of the active substance must be available.

In the case of micro-organisms state methods of detection, enumeration, identification and relevant markers.

SECTION III

STUDIES CONCERNING THE EFFICACY OF THE ADDITIVE

1. Studies concerning improvements in the characteristics of feedingstuffs.

These studies concern technological additives such as antioxidants, preservatives, binders, emulsifiers, stabilizers and gelling agents, which are intended to improve or stabilise the characteristics of premixtures and feedingstuffs. Some micro-organisms and/or enzyme preparations could also be considered as technological additives, if they improve relevant feed characteristics.

Evidence of the efficacy of the additive should be provided by means of appropriate criteria under the intended conditions of use in comparison with negative control feedingstuffs and, possibly, feedingstuffs containing technological additives of known effectiveness.

The precise nature of the active substances, preparations, premises and feedingstuffs examined, the reference number of the batches, the concentration of the active substances in premixtures and feedingstuffs, the testing conditions (e.g. temperature and humidity) and also the dates and duration of testing, the adverse and negative effects which occurred during testing shall be specified for each experiment.

2. Studies concerning the effects of additives on animal production.

These studies concern zootechnical additives which have effects on animal production. The following studies including dose-response relationship, should be performed on each target species in comparison with negative control groups and, possibly, groups

receiving feedingstuffs containing additives of known effectiveness. If the active substance is a mixture of effective components, the presence of each component must be justified.

2.1. For coccidiostats and other medicinal substances, importance should primarily be attached to evidence of the specific effects and particularly prophylactic properties (e.g. morbidity, oocyst count and lesion score). Information on the effect on feed efficiency, animal growth and marketable quantity and quality of the animal produce may be added.

2.2. For other zootechnical additives (including micro-organisms and/or enzyme preparations) information should be provided on the effects on: nutritional efficiency, animal growth, animal products characteristics and yield, animal welfare and other parameters having a positive influence on animal production.

2.3. Experimental conditions :

The test performed must be described and the results presented individually in detail. The statistical evaluation and the methods employed should be reported. The following data must be provided :

2.3.1. Species, breed, age and sex of the animals, identification procedure.

2.3.2. Number of test and control groups, number of animals in each group. The number of animals of both sexes must be sufficient for statistical purposes.

2.3.3. Concentration of the active substance using the appropriate recognized measure in the feedingstuffs established by a control analysis. Reference number of the batches. Nutritional composition of the diet in terms of quality and quantity.

2.3.4. Location of each experiment. Animal health, physiological, feeding and rearing conditions as usually practiced in the Community. Feed control and measures taken to avoid contamination of control groups during the experiment (particularly for micro-organisms through self contamination by the micro-organism).

2.3.5. Date and exact duration of testing.Date and nature of the examinations performed

2.3.6. Unfavourable effects and other incidents which occurred during the experiment and time of their appearance.

3. Studies concerning the quality of animal produce.

Studies on the organoleptic, nutritional, microbial, hygienic and technological qualities of edible produce from animals fed with feedingstuffs containing the additive. Studies of characteristics of the animal produce and effects on the final composition should be provided.

SECTION IV

STUDIES CONCERNING THE SAFETY OF USE OF THE ADDITIVE

The studies outlined in this section are intended to permit assessment of :

- the safety of use of the additive in the target species,
- the risks from inhalation or from cutaneous, mucosal or eye contact for persons likely to handle the additive as such or as incorporated into premixtures of feedingstuffs,
- the risks to the consumer which could result from the consumption of food containing residues of the additive, or its metabolites,
- the risks of pollution or survival of the environment from the additive itself or by products derived from the additive and excreted by animals,
- the risks to non-target species.

These studies will be required in their entirety or in part depending on the nature of the additive and the conditions proposed for its use; in the case of micro-organisms and/or enzyme preparations appropriate safety tests must be performed (including tolerance tests and a translocation test). If the active substance is chemically specified the knowledge of their metabolism in the various target species and also of the composition and the bioavailability of the tissue residues will be essential for determining the extent of studies on laboratory animals to assess the risks for the consumer. Furthermore, knowledge of the composition and of the physico-chemical and biological properties of the excreted residues deriving from the additive will be indispensable to define the extent of the studies necessary for assessment of the risk of pollution or survival of the environment.

1. Studies on target species

1.1. Toxicological studies of the additive.
Tolerance tests.
Study of the biological, toxicological, macroscopic and histological effects. Determination of the safety margin between the maximum proposed dose-level and the level resulting in unfavourable effects. It may be sufficient to indicate a minimum or approximate value for this margin if it can be shown that the level resulting in unfavourable effects greatly exceeds the maximum proposed dose-level.

1.2. Microbiological studies of the additive.

1.2.1. If the active substance is chemically specified studies of the antimicrobial spectrum of action of the additive by determination of the Minimum Inhibitory Concentration (MIC) in various pathogenic and non-pathogenic Gram-negative and Gram-positive species of bacteria should be provided.

1.2.2. Studies on the cross-resistance to therapeutic antibiotics by determination of the MIC in mutants produced, which exhibit chromosomal resistance to the additive. In the case of the use of micro-organisms which are resistant to therapeutic antibiotics a plasmid profile should be provided.

1.2.3. Tests to find out whether the additive is capable of selecting resistance factors. These tests are to be performed under field conditions in the animal species for which the additive is primarily intended. Subsequently, it should be determined whether R factors which may have been found carry multiple resistance and are transmissible.

1.2.4. Tests to determine the effect of the additive

- on the microflora of the digestive tract
- on the colonisation of the digestive tract
- on the shedding or excretion of pathogenic micro-organisms.

1.2.5. If the active substance shows an antimicrobial action field studies to monitor the percentage of bacteria resistant to the additive should be provided. These are to be carried out at major intervals before, during and after the use of the additive.

1.2.6. If the additive is a micro-organism, it should be determined if it is resistant to antibiotics.

1.2.7. If the additive is a genetically modified micro-organism, the specifically adopted guidelines should be followed .

1.2.8. If the additive (e.g. enzyme preparations) is produced by a micro-organism the level of contamination by the viable producer organism (if any) should be determined.

1.3 Studies of the metabolism and residues(1)(2)
(when the active substance is chemically specified)

1.3.1. Study of metabolism

- metabolic balance: rate and extent of absorption and elimination of the active substance,
- identification of the metabolic pathways and main metabolites,
- distribution and excretion (billiary, urinary, faecal) of the metabolites,
- if appropriate influence of the intestinal or ruminal microflora, of enterohepatic cycle, of caecotrophy, on the metabolism.

(1) The studies mentioned under 1.3.1., 1.3.3., and 1.3.4. should be carried out with labelled molecules or other appropriate methods, in each case the choice of the method utilised should be justified. The labelling shoud be suitable for the purpose intended.

(2) If the active substance is produced by fermentation, these studies should be extended to related substances derived from the production.

1.3.2. Analytical studies of the residues : qualitative and quantitative composition of the residues (active substance, metabolites) in the various animal food products at metabolic equilibrium and under practical conditions of use of the additive.

1.3.3. Kinetic study of the residues (following repeated administration of the additive according to the proposed use) : persistence of the active substance and the main metabolites in the various organs and tissues after withdrawal of the supplemented feedingstuff.

1.3.4. Study of the bioavailability of the residues in animal food products before and after storage and cooking (see 3.7.).

1.3.5. Methods of monitoring : qualitative and quantitative methods of determination used in the studies mentioned under items 1.3.1. to 1.3.4. with information as to percentage recovery, specificity and limits of detection. The methods of determination of the residues must be sufficiently sensitive to permit detection of residues at levels which are toxicologically negligible.

2. Study on excreted residues
(when the active substance is chemically specified)

2.1. Nature and concentration of the residues derived from the additive (active substance, metabolites) in the excreta.

2.2. Persistence (half-life value) and kinetics of elimination of these residues in slurries, farm yard manure and litter.

2.3. Effects on methanogenesis.

2.4. Degradation, persistence (half-life value) and kinetics of elimination in soils (contrasting soil types).

2.5. Effects on soil fauna and microbial processes of transformation (e.g. decomposition of plant and animal residues).

2.6. Effects on terrestrial plants (e.g. seed germination, plant growth and plant up-take). These studies should be carried out under controlled conditions and field conditions, using different plant species.

2.7. Solubility and stability in water of the products derived from the additive (active substance, metabolites).

2.8. Effects on aquatic life.

2.8.1. Effects on flora (e.g. *Chlorella*).

2.8.2. Toxicity in non-vertebrates (*e.g. Daphnia magna*).

2.8.3. Toxicity in fish (at least two wild species found in the Community territory).

3. Studies on laboratory animals
(when the active substance is a non pathogenic micro-organism found naturally these type of studies may not be necessary).

These studies must be carried out with the active substance and its major metabolites or products, if the latter are also present in edible animal produce and are bioavailable. As far as possible attempts should be made to select laboratory animals which may be expected to digest and metabolize the additive in a similar way to man or the target species.

Full detailed descriptions must be provided of the tests performed. These should cover the animal species and strains employed, the size and number of test and control groups, the dose levels administered, the composition of the diet and the results of feed analyses, the rearing conditions, the exact duration of the tests, the dates of the various examinations performed and mortality. Full details must be given of the macroscopic pathological and histopathological findings in all animals tested

with an indication of the time of appearance of all pathological lesions. All results, including statistical assessment, must be presented in detail.

3.1. Acute toxicity

3.1.1. Acute oral toxicity studies must be carried out on two animal species (preferably the rat should be one). The maximum dosage should not be higher than 2,000 mg/kg body weight. Detailed observations should be reported of the biological effects observed during a period of at least two weeks after ingestion.

3.1.2. Studies on acute inhalational toxicity, skin and, where necessary, mucous membranes irritancy and also allergenic potential must be performed by appropriate tests for the assessment of possible risks associated with the handling of the additive.

3.2. Mutagenicity

In order to identify active substances or their metabolites or products that posses mutagenic properties a selected combination of mutagenicity tests, based on different genetic endpoints, must be carried out, except if the active substance is a micro-organism. Tests must be performed, in the presence and absence of a microsomal mammalian preparation for a metabolic activation.

The following package of tests is recommended :

(a) a test for gene mutations in a prokaryotic system,

(b) a test for gene mutations in an *in vitro* eukaryotic system or a sex-linked recessive lethal test in *Drosophila melanogaster*,

(c) a test for chromosomal damage *in vitro* and *in vivo*.

The battery of tests suggested above does not imply, however, that other tests are inappropriate or that other tests, in particular *in vivo* tests, would not be acceptable as alternatives.

In all cases reasons for the choice of tests should be given. Tests must be carried out according to established validated procedures. Depending on the outcome of the tests and taking into consideration the whole toxicity profile of the substance as well as the intended use, additional investigations may be indicated.

3.3. Pharmacokinetic aspects

If the active substance is chemically specified balance studies and identification of metabolites must be performed using suitable labelled molecules or other appropriate techniques and should cover both single and multiple dose administration of the active substance over appropriate periods. Metabolism studies must also include investigation of the pharmacokinetics of the active substance and of the major metabolites. Consideration must be given to the differences in the way that various species metabolize the active substance when selecting the most relevant species for subsequent toxicological investigations.

3.4. Subchronic toxicity

These studies must be carried out in general on two animal species (preferably the rat should be one). The second species may in some instances be a target species. The test substance may be administered orally and a dose-response relationship must be established. The duration in rodents must be at least 90 days.

In certain cases investigations extending over six months to two years in non-rodents (the commonly used non-rodent is the dog, preferably of a defined breed) may be desirable to establish the variation in sensitivity of different animal species to the test substance.

These studies are not relevant for micro-organisms, in the case of enzymic preparations appropriate tests should be provided.

3.5. Chronic toxicity/carcinogenicity

Chronic toxicity studies must be carried out on one species (preferably the rat), carcinogenicity studies preferably on two species of rodent. The substance must be administered orally at several dose levels. A combined chronic toxicity/carcinogenicity study with in-utero exposure is also acceptable. Experiments must extend for a minimum of 24 months in rats and 18 months in mice. If continued beyond the minimum period, the test must be terminated when survival in any but the highest dose level groups has fallen to 20%.

Full clinical chemistry, haematological and urine examinations must be carried out at appropriate intervals throughout the experiment. Full macroscopic and histological examinations must be carried out on all animals dying during the test and on all survivors at the termination of the study.

These studies are not relevant for micro-organisms, in the case of enzymic preparations appropriate tests should be provided.

3.6. Reproductive toxicity

Studies on reproduction must be carried out preferably on the rat. They must extend over at least two filial generations and may be combined with embryotoxicity including teratogenic studies. All relevant fertility, gestation, parturition, peri- and postnatal parameters must be carefully observed and reported. Specific teratogenic studies must be carried out in at least two suitable species.

3.7. Toxicology of metabolites

Information for the calculation of residue concentration is required as a basis for assessing the risk for man.
The basis for calculation of the proposed withdrawal period must be made available. The studies mentioned in 1.3.4. must be carried out in laboratory animals.

3.8. Other relevant studies.

Any further special study providing additional information useful for the assessment of test substance may be made available (e.g. bioavailability, neurotoxicity or immunotoxicity).

SECTION V

FORM OF MONOGRAPH

1. Identity of the additive

1.1. Type of additive according to its main function

1.2. Qualitative and quantitative composition (active substance, other components, impurities)

1.3. Physical state, particle size

1.4. Possible specific processing.

N.B. If the active substance is a mixture of active components, each clearly definable, main components must be described separately and the proportions of the mixture given.

2. Specifications concerning the active substance

2.1. Generic name, chemical name according to IUPAC nomenclature, other non-proprietary and generic international names and abbreviations. Chemical Abstracts Service Number (CAS).

If the active substance is a micro-organism: name and taxonomic description according to the international Codes of Nomenclature. Other internationally recognized Manuals of Systematics can also be used(10).

For enzyme preparations : name according to main enzymic activities as described by IUB/IUPAC, EINECS and CAS Number.

2.2. Formula, empirical and structural, and molecular weight. Qualitative and quantitative composition of the main components, if the active substance is a fermentation product.

For micro-organisms : name and place of culture collection, if possible one in an EEC collection, where the strain is deposited

(10) Such as Bergey's Manual of Determinative Bacteriology. "The Yeasts, a taxonomic study" by Lodder, "A Dictionary of Fungi" by Ainsworth and Bisley or "The Genus Aspergillus" by Raper and Fennel.

and depositing number, genetic modification and all relevant properties for its identification.

For enzyme preparations : the biological origin, the activities towards relevant chemically pure model sustrates and other physico-chemical characteristics.

2.3. Degree of purity

For micro-organisms : genetic stability and purity of strains cultivated.
For enzyme preparations : purity (checking the level of contaminating micro-organisms, heavy metals, absence of mycotoxins and antibacterial activity),and composition of the non-enzymic component derived from the source material.

Qualitative and quantitative composition of the impurities

2.4. Relevant properties

For chemicals: Electrostatic properties, melting point, boiling point, decomposition temperature, density, vapour pressure, solubility in water and organic solvents, mass and absorption spectra and any other appropriate physical properties.

For micro-organisms : relevant biological properties.
For enzyme preparations: optimal pH, temperature and other appropriate properties.

3. Physico-chemical, technological and biological properties of the additive.

3.1. Stability (for micro-organisms loss of biological activity, e.g. viability) on exposure to environmental conditions such as light, temperature, pH, moisture and oxygen. Expiry date.

3.2. Stability (for micro-organisms loss of biological activity, e.g. viability) during the preparation of premixtures and feedingstuffs, in particular stability to heat, pressure and moisture. Possible decomposition products.

3.3. Stability (for micro-organisms loss of biological activity, e.g. viability) during the storage of premixtures and feedingstuffs (storage time under defined conditions). Expiry date.

3.4. Other appropriate physico-chemical, technological or biological properties such as ability to obtain homogeneous mixtures in premixtures and feedingstuffs, dust-forming properties, and for micro-organisms and/or enzyme preparations, assessment of resistance to degradation or loss of biological activity in the digestive tract or by systems of simulation *in vitro*.

3.5. Physico-chemical or biological incompatibilities or interactions (e.g. with feedingstuffs, other additives or with medicinal products).

4. Control methods

4.1. Description of the methods used for the determination of the criteria listed under items 1.2., 2.3., 2.4., 3.1., 3.2., 3.3. and 3.4. of this Section.

4.2. Description of the qualitative and quantitative analytical methods for determining, residues of additives in animal produce.

4.3. If the said methods have been published, literature references may suffice, in this case reprints should be provided.

5. Biological properties of the additive

5.1. Particulars of the prophylactic effects for coccidiostats and other medicinal substances (e.g. morbidity, oocyst count and lesion score).

5.2. For zootechnical additives other than those listed in 5.1.(including micro-organisms and/or enzyme preparations. Particulars of the effects on feed efficiency, animal growth, animal products characteristics and yield, animal welfare and other parameters having a positive influence on animal production.

5.3. Any contra-indications or warnings, including biological incompatibilities, with particulars of their justification.

6. Details of the quantitative and qualitative residues, if any, found in animal produce following envisaged use of the additive.

7. Other characteristics suitable for identification of the additive.

REPORTS OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION
ACCUMULATIVE INDEX, 1979–1992

First Series (1979)
Catalogue No CB-28-79-277-EN-C

(1) Dimetridazole (Turkey, Guinea-Fowl & Swine); Ipronidazole (Turkeys & Guinea-Fowl), Ronidazole (Turkey & Swine).

(2) Oleandomycin (Turkeys, Other poultry, Swine), Spiramycin (Turkeys, Other poultry, Swine, Calves, Lambs & Kids, Animal breed for fur), Virginiamycin (Turkeys, Other poultry, Swine, Calves, Lambs & Kids, Animal breed for fur), Erytromycin (Chicken for fattening, swine), Tylosin (Swine), Lyncomicin (Poultry) Oleandomycin (Poultry, Swine), Spiramycin (Poultry, Swine, Calves, Lambs & Kids, Animal breed for fur), and Virginiamycin (Poultry, Swine, Calves).

Second Series (1980)

Report 6918 EN. Catalogue Nº CDE-NK-80-002-EN-C

1. Report of the Scientific Committe for Animal Nutrition on the use of **Carbadox** in feedingstuffs for pigs. Opinion expressed: 6 July 1978. p. 7.

2. Report of the Scientific Committee for Animal Nutrition on the use of **Halofuginone** in feedingstuffs for **Chickens**. Opinion expresed: 25 April 1979. p. 11.

3. Report of the Scientific Committee for Animal Nutrition on the use of **Monensin Sodium** and **Flavophospholipol** in feedingstuffs for **Fattening Cattle**. Opinion expressed 26 April an 11 July 1979. p. 14.

4. Report of the Scientific Committee for Animal Nutrition on the use of **Avoparcin** in feedingstuffs for **Chickens** and **Pigs**. Opinion expressed: 11 July 1979. p. 22.

5. Report of the Scientific Committee for Animal Nutrition on the **guide-Lines** for the assessment of **additives** in feedingstuffs. p. 25.

Third Series (1981)

Report EUR 7383

1. Report of the Scientific Committee for Animal Nutrition on the use of **Fumaric** and **Malic** acids in feedingstuffs. Opinion expressed: 15 January 1980. p. 5.

2. Report of the Scientific Committee for Animal Nutrition on the use of **Hydrochloric** and **Sulphuric** acids in feedingstuffs. Opinion expressed: 15 January 1980. p. 8.

3. Report of the Scientific Committee for Animal Nutrition on the use of **Polyethylene Glycol 6000** and of a **Polyoxypropylene-Polyoxyethylene polymer** in feedingstuffs. Opinion expresed: 15 January 1980. p. 12.

4. Report of the Scientific Committee for Animal Nutrition on the use of **Arprinocid** in feedingstuffs for **chickens**. Opinion expressed: 11 December 1980. p. 15.

5. Report of the Scientific Committee for Animal Nutrition on the use of inactivated **cultures** of selected enteropathogenic strains of **E. coli** in feedingstuffs for **Piglets**. Opinion expressed: 11 March 1981. p. 18.

6. Report of the Scientific Committee for Animal Nutrition on **Guidelines** for the assessment of **certain products** to be used as sources of proteins in animal nutrition. Opinion expressed: 11 March 1981. p. 22.

Fourth Series (1984)

Report EUR 8769. Catalogue No CD-NK-83-010-EN-C

1. Report of the Scientific Committee for Animal Nutrition on the use of **Olaquindox** in the feedingstuffs for **Pigs**. Opinion expressed: 8 July 1981. p. 1.

2. Report of the Scientific Committee for Animal Nutrition on the use of **Copper Methionate** in feedingstuffs for **Pigs**. Opinion expressed: 7 October 1981. p. 6.

3. Report of the Scientific Committee for Animal Nutrition on the use of **Lincomycin**(3) and **Spiramycin** in feedingstuffs. Opinion expressed: 7 October 1981. p. 9.

4. Report of the Scientific Committee for Animal Nutrition on the use of **Monensin Sodium** in feedingstuffs for **Poultry**. Opinion expressed: 10 February 1982. p. 31.

5. Report of the Scientific Committee for Animal Nutrition on the use of **Robenidine** in feedingstuffs for **Rabbits**. Opinion expressed: 10 February 1982. p. 31.

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(3) Lincomycin (Poultry). Spiramycin (Turkeys, Other poultry, Pigs, Piglets, Calves, Lambs, Kids, Fur-animals).

6. Report of the Scientific Committee for Animal Nutrition on the use of **Salinomycin** in feedingstuffs. Opinion expressed: 14 April 1982(4). p. 36.

7. Report of the Scientific Committee for Animal Nutrition on the use of **Narasin** in feedingstuffs for **chickens**. Opinion expressed: 14 April 1982. p. 41.

8. Report of the Scientific Committee for Animal Nutrition on the use of **Nifursol** in feedingstuffs for **Turkeys**. Opinion expressed: 14 April 1982. p. 46.

9. Report of the Scientific Committee for Animal Nutrition on the use of **Nicarbazin** in feedingstuffs for **Poultry**(5). Opinion expressed: 14 April 1982. p. 51.

10. Report of the Scientific Committee for Animal Nutrition on the use of **Copper** compounds in feedingstuffs(6). Opinion expressed: 15 April 1982. p. 56.

11. Second report of the Scientific Committee for Animal Nutrition on the use of **Carbadox**(7) in feedingstuffs for **Pigs**. Opinion expressed: 7 July 10 1982. p. 82.

12. Report of the Scientific Committee for Animal Nutrition on the use of **Lerbek**(8) in feedingstuffs for **Poultry**. Opinion expressed: 17 November 1982. p. 87.

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(4) Chickens, Piglets, Pigs and Cattle. Provisional pending on reassesment.
(5) Authorized only for fattening chickens.
(6) Piglets, Pigs, Sehep and other animal species.
(7) This report on carcinogenic potential of carbadox and its metabolites. First report on 2nd Series, p. 7.
(8) Registered trademark of premix of metilclorpindol and methylbenzoquate (MBQ) (100/8.35 W/W).

13. Report of the Scientific Committee for Animal Nutrition on the use of **Halofuginone** in feedingstuffs for **Turkeys**[9]. Opinion expressed: 17 November 1982. p. 94.

14. Report of the Scientific Committee for Animal Nutrition on the use of **Virginiamycin** in feedingstuffs for **Laying hens**[10]. Opinion expresed: 17 November 1982. p. 98.

15. Report of the Scientific Committee for Animal Nutrition on the use of **Canthaxanthin** in feedingstuffs for **Salmon** and **Trout**. Opinion expressed: 14 December 1982. p. 102.

16. Report of the Scientific Committee for Animal Nutrition on the use of **Lasalocid Sodium** in feedingstuffs for **Chickens**. Opinion expressed: 14 December 1982. p. 106.

17. Report of the Scientific Committee for Animal Nutrition on the use of **Formaldehyde** in feedingstuffs for **Piglets**[11]. Opinion expressed: 20 April 1983. p. 111.

18. Report of the Scientific Committee for Animal Nutrition on the use of **Copper** compounds in feedingstuffs for **Pigs**[12][8]. Opinion expressed: 1 June 1983. p. 116.

19. Report of the Scientific Committee for Animal Nutritionon the use of **Avoparcin** in feedingstuffs for **Calves** and **Fattening Cattle**. Opinion expressed: 1 June 1983. p. 120.

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(9) No objections to the use, however unable to issue a final opinion before the results the additional mutagenecicity test requested.

(10) Request additional data to justufy dose of 40 mg/kg.

(11) Recommends precautions in handling.

(12) Piglets and pigs, see former opinion in 4th Series, p.56 (15 April 1982).

Fifth Series (1986)

Report EUR 1041EN. Catalogue No CD-NK-86-003-EN-C

1. Second report of the Scientific Committe for Animal Nutrition on the use of **Monensin Sodium** in feedingstuffs for **Turkeys**[13]. Opinion expressed: 23 November 1983. p. 1.

2. Second report of the Scientific Committee for Animal Nutrition on the use of **Narasin**[14] in feedingstuffs for **Chickens**. Opinion expressed: 8 February 1984. p. 4.

3. Third report of the Scientific Committee for Animal Nutrition on the use of **Halofuginone** in feedingstuffs for **Poultry**[15]. Opinion expressed: 8 February 1984: p. 6.

4. Second report of the Scientific Committee for Animal Nutrition on the use of **Olaquindox** in feedingstuffs for **Pigs**[16]. Opinion expressed: 3rd May 1984. p. 11.

5. Report of the Scientific Committee for Animal Nutrition on the use of **Pancoxin**[17] and **Pancoxin Plus** in feedingstuffs for **Poultry**. Opinion expressed: 3 May 1984. p. 13.

6. Second report of the Scientific Committee for Animal Nutrition on the use of **Lerbek**[18] in feedingstuffs for **Turkeys**[19]. Opinion expressed: 11 July 1984. p. 23.

7. Report of the Scientific Committee for Animal Nutrition on the use of **Zinc Bacitracin** in feedingstuffs for **Poultry, Pigs** and **Rabbits**. Opinion expressed: 30 January 1985. p. 25.

(13) First report in 4th Series, p.25.
(14) First report in 4th Series, p.41.
(15) First report in 2nd Series, p.11. Second report in 4th Series, p. 94.
(16) First report in 4th Series, p.1.
(17) Mixture of 18 parts amprolium, 10,8 sulphaquinoxaline, 0,9 ethopabate.
(18) Registered trade mark of a premix containing 100 parts of meticlorpindol and 8.35 partes of methylbenzoquate.
(19) First report in 4th Series. p. 87.

8. Second report of the Scientific Committee for Animal Nutrition on the use of **Nicarbazin** in feedingstuffs for **Fattening Chickens**[20]. Opinion expressed: 5 July 1985. p. 33.

9. Report published jointly by the Scientific Committee for Animal Nutrition and the Scientific Committee for Food on the use in animal nutrition of protein products obtained from **Candida yeasts**. Opinion expressed: 3 May 1984. p. 35.

10. Report published jointly by the Scientific Committee for Animal Nutrition and the Scientific committee for food on the use in animal nutrition of protein products obtained from bacteria of the **Metilomonadaceae family**[21]. Opinion expressed: 23 September 1985. p. 51.

Sixth Series

Report EUR 10996. Catalogue Nº CD-NA-12824-EN-C

1. Report of the Scientific Committee for Animal Nutrition on the use of **Flavophospholipol** in feedingstuffs[22] for **Rabbits**. Opinion expressed: 22 January 1986. p. 1.

2. Report of the Scientific Committee for Animal Nutrition on the use of **Nicarbazin** in feedingstuffs[23] for **Chickens**. Opinion expressed: 8 July 1986. p. 5.

3. Report of the Scientific Committee for Animal Nutrition on **Guidelines** for the Assesment of **Additives** in Feedingstuffs[24]. p. 8.

(20) First report in 4th Series, p.51.

(21) In particular from Methylophilus cultivate in methanol (Pruteen).

(22) Opinions given for laying hens (see 1st Series pp. 21 & 27); and Fattening Cattle (see 2nd. Series p.14).

(23) Opinions given for laying hens (see 1st Series pp. 21 & 27); and Fattening cattle (see 2nd Series p.14).

(24) In regards to Article 7 (2) of Council Directive 70/524/EEC (O.J. Nº L270, 14.12.1970 p.1. & O.J. Nº L319, 08.12.1984, p.13).

Seventh Series

Report EUR 12824 EN Catalogue No CD-NA-12824-EN-C

1. Report of the Scientific Committee for Animal Nutrition on the use of **Maduramicin Ammonium** in feedingstuffs for **Chickens** for fattening. Opinion expressed: 27 April 1988. p. 1.

2. Second report of the Scientific Committee for Animal Nutrition on the use of **Flavophosopholipol** in feedingstuffs for **Rabbits**[25]; Opinion expressed: 27 April 1988. p. 13.

3. Report of the Scientific Committee for Animal Nutrition on the use of **Avilamycin** in feedingstuffs for **Pigs**. Opinion expressed: 27 April 1988. p. 13.

4. Report of the Scientific Committee for Animal Nutrition on the use of **Virginiamycin** in feedingstuffs for **Cattle**[26]. Opinion expressed: 27 April 1988. p. 21.

5. Report of the Scientific Committee for Animal Nutrition on the use of **Asxtaxanthin** in feedingstuffs for **Salmon** and **Trout**. Opinion expressed: 8 March 1989. p. 29.

6. Report of the Scientific Committee for Animal Nutrition on the use of **Nitrovin** in feedingstuffs[27]. Opinion expressed: 29/30 November 1988. p. 33.

(25) Opinions for Laying hens (1st Series, pp.: 21 & 27), Fattening cattle (2nd. Series, p.14) already given.

(26) Opinion for utilization in Turkeys, Swine and Calves in 1st Series p.15. Opinion for use in laying hens in 4th Series p.98.

(27) Provisional opinion pending on salmonella shedding experiment.

Eight Series
(In preparation)

1. **Report of the Scientific Committee for Animal Nutrition on the use of Lasalocid Sodium** in feedingstuffs for **Cattle**. Opinion expressed: 27 July 1990. p. 3

2. Report of the Scientific Committee for Animal Nutrition on **Efrotomycin** the use of in feedingstuffs for **Pigs**. Opinion expressed: 27 July 1991. p. 11

3. Report of the Scientific Committee for Animal Nutrition on the use of **Lasalocid Sodium** in feedingstuffs for **Turkeys**. Opinion expressed: 10 July 1991. p. 19

4. Report of the Scientific Committee for Animal Nutrition on the use of **Salinomycin** in feedingstuffs for **Pigs**. Opinion expressed: 10 July 1991. p. 23.

5. Report of the Scientific Committee for Animal Nutrition on the use of **Narasin/Nicarbazin** in feedingstuffs for **Chickens**. Provisional opinion expressed: 10 July 1991. p. 29.

6. Report of the Scientific Committee for Animal Nutrition on the use of **Diclazuril** in feedingstuffs for **Chickens**. Opinion expressed: July 1991. p. 39.

7. Report of the Scientific Committee for Animal Nutrition on the use of **Propylene-glycol** (1,2-Propanediol) in feedingstuffs for **Cats**. Provisional opinion expressed: 15 January 1991. p. 45.

8. Amendments proposed to modify the Guidelines for the Assessment of Additives in Animal Nutrition[28] to make this document also applicable for micro-organisms and/or enzyme preparations intended for nutritional purposes: P.: 49

9. Reports of the Scientific Committee for Animal Nutrition. Cumulative index 1974 - 1992.

(28) Council Directive 87/153/EEC, O.J. No. L64 (7.3.87) p.19.

European Commission

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Eighth series — 1992

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