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

Opinion expressed 3 May 1984

TERMS OF REFERENCE (April 1981)

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

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

2. Could the use of these additives affect the development of resistance in bacteria ?

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

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

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning Additives in feedingstuffs (1), as last amended by the thirty seventh Commission Directive of 9 April 1981 (2),

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(*) Registered trade name
(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 131, 18.05.1981, p. 1

Member States are authorized by way of derogation to use Pancoxin (*) and Pancoxin Plus (*) up to the 30 November 1981 under the following conditions set out in Annex II, Section B, of the Directive :

Additive	Species of animal	Min. content: ppm (mg/kg) of complete feedingstuffs	Max. content: ppm (mg/kg) of complete feedingstuffs	Other provisions
Pancoxin (*) : mixture of 18 parts amprolium (a), 10.8 parts sulphaquinoxaline (b) and 0.9 parts ethopabate (c)	Chickens for fattening, turkeys	-	(a): 100 (b): 60 (c): 5	Use prohibited at least seven days before slaughter
Pancoxin Plus (*) : mixture of 20 parts amprolium (a), 12 parts sulphaquinoxaline (b), 1 part ethopabate (c) and 1 part pyrimethamine (d)	Chickens for fattening	-	(a): 100 (b): 60 (c): 5 (d): 5	Use prohibited at least seven days before slaughter

OPINION OF THE COMMITTEE

The Committee noted that the studies on metabolism, residues and excreted products from Pancoxin (*) and Pancoxin Plus (*), available in 1981, were insufficient and requested that further extensive studies be carried out. As supplementary dossiers were now available, the Committee expressed the following opinion.

1. The individual constituents (amprolium, sulphaquinoxaline, ethopabate and pyrimethamine) of Pancoxin (*) and Pancoxin Plus (*) have been examined toxicologically but not all substances have been studied to

the same extent. No toxicity or residue data are available on chickens and turkeys fed on Pancoxin (*). Metabolism studies are not available for pyrimethamine in chickens and for amprolium and ethopabate in turkeys. Residue data are available only for chickens fed on amprolium, sulphaquinoxaline and ethopabate or Pancoxin Plus (*) and for turkeys fed on sulphaquinoxaline. No information was supplied on the nature of the residues for any of the constituent substances. Therefore, the Committee considered that the available information summarised below is still insufficient to reply fully to question No 1 put by the Commission. It appeared however that residue data of sulphaquinoxaline point to the need for a withdrawal period of at least 7 days in chickens and at least 14 days in turkeys.

1.1. Amprolium

Metabolism was studied in chicken using orally administered ^{14}C radiolabelled material. Over 90% of radioactivity was excreted in 48 hours, about 75% being unchanged amprolium.

Residues in chicken fed 125-250 mg/kg in the feed for up to 8 weeks were highest in kidneys but low in liver and muscle. After 3 weeks feeding, residues ranged from 1-3 mg/kg in kidneys, 0.7-1.7 mg/kg in the liver to 0.2 mg/kg in muscle. After 4 days withdrawal, residues were less than 0.01 mg/kg in all tissues (limit of detection 0.01-0.02 mg/kg).

Toxicity studies were performed in mice, rats and dogs. The oral LD_{50} in mouse and rat was 4 mg/kg b.w. Ninety-day studies in rats and dogs produced weight loss, diarrhoea and increased mortality with the no-effect-level being 200 and 400 mg/kg b.w. A two year study in the rat was inadequate to determine a no-effect-level but showed no carcinogenic effect. High doses reduced growth and survival. The two year dog study suggests a no-effect-level of 100 mg/kg b.w. No reproduction or teratology studies were available. Mutagenicity tests in prokaryotic systems were negative.

1.2. Sulphaquinoxaline

Studies of the metabolism in chickens and turkeys showed rapid absorption with dose-dependent plasma levels persisting for up to 12 hours. Chickens acetylate the compound to a small degree. Most species form the insoluble 3-hydroxy derivative which is deposited as renal crystals. There is little information on metabolites. Sulphaquinoxaline diffuses into eggs in proportion to the plasma levels.

Broilers treated for 2 weeks with a subsequent 7-day withdrawal period had residues in the skin of 0.14 mg/kg and in liver, kidneys, muscle and fat below 0.1 mg/kg (sensitivity of method 0.1 mg/kg). Residues in cockerels treated for 2 weeks followed by a 7-day withdrawal period were 0.28 mg/kg in kidneys, 0.1 mg/kg in the liver, 0.11 mg/kg in the skin and less than 0.1 mg/kg in muscle and fat. Residues in the liver, kidneys, muscle and skin of turkeys were below 0.15 mg/kg after 10-14 days withdrawal periods. Broilers treated for 43 days with Pancoxin Plus (*) (60 mg/kg feed sulphaquinoxaline) had no detectable residues after a 5-day withdrawal period (sensitivity of method not stated).

The toxicity has been studied in several species. The oral LD_{50} for mice is 15 g/kg b.w., for rats 1 g/kg b.w. The main toxic effects are interference with blood clotting mechanism, tubular nephropathy with renal crystal deposition and focal hepatic necrosis. A 90-day study in rats showed enlargement of the thyroid with a no-adverse effect level of 2 mg/kg b.w., but longer studies at high levels produced tubular nephropathy, enlargement of the thyroid and testicular atrophy. A 90-day study in beagle dogs gave similar results with a no-effect-level of less than 2 mg/kg b.w. A large number of short-term studies in chicken and turkeys produced haemopoietic effects at high doses but 120-500 mg/kg b.w. were tolerated over several months. A long-term study, multigeneration reproduction study and

teratology study in rats showed no abnormal effects at dose levels of 0.38-2.5 mg/kg b.w. The mutagenicity studies in prokaryotic systems were negative.

1.3. Ethopabate

The metabolism was studied in rats, dogs and chicken. Single oral doses were almost entirely excreted within 24 hours in the urine of all species, almost all as unchanged etho- pabate. Chicken excreted 87-100% of oral single doses and 0.07-11% as CO_2, determined by the use of radiolabelled ma- terial. The proportion of radioactivity exhaled depended on the position of radiolabel. No metabolites or ethopabate were dete- cted in the tissues. The metabolite 4-acetylamino-2-ethoxyben- zoic acid was identified in the urine of chicken.

Residue studies in chicken with repeated doses showed the highest levels when the aromatic ring was labelled. Levels in the kidneys were 1.3 mg/kg, in the liver 0.9 mg/kg and in muscle 0.2 mg/kg. After 5 days withdrawal residues were 0.05 mg/kg in all tissues (sensitivity of method 0.05 mg/kg). If the ethoxy group was labelled, residues ranged from 0.05-0.3 mg/kg depending on dose level and no radioactivity was detectable after 8 hours in any tissue. If the carboxy group was labelled, residues were 0.3 mg/kg in muscle, 1 mg/kg in kidneys and 0.6 mg/kg in the liver, reducing to 0.05 mg/kg or less after 5 days withdrawal.

Toxicity studies include oral LD_{50} in mouse and rat (about 14 g/kg b.w.) and short-term studies in rat, dog and chicken. Rat and dog studies with doses ranging from 10 mg/kg to 5 g/kg b.w. pro- duced mainly hepatotoxicity, the no-effect-level for the dog being 10 mg/kg b.w. and for the rat 100 mg/kg b.w. Feed intake, egg production and hatchability in chicken were adversely affec- ted by 500 mg/kg in the feed.

Long-term studies in rats and dogs showed only hepatotoxic effects but no carcinogenic potential. No reproduction or teratology studies were available. Mutagenicity studies in prokaryotes and Saccharomyces were negative.

1.4. Pyrimethamine

Metabolic studies in mice, rabbits and monkeys show that 50-60% of single oral doses are excreted in the faeces within 24 hours. Man excretes pyrimethamine slowly, about 12% of the dose appearing in the urine within 5 days but excretion is still detectable in the urine for 11 days and in the blood for 7 days.

The toxicity has been studied in several species. The oral LD_{50} for mice is 90 mg/kg b.w. Short-term studies extending over 42 to 90 days in rats, dogs and monkeys showed the main toxic effect to be bone marrow depression together with other species specific adverse effects on growth, testes, kidney and the gastrointestinal tract. The no-adverse-effect level varied from 1.25 mg/kg b.w. in monkeys and dogs to 2.5 mg/kg b.w. in rats. Chickens showed similar bone marrow disturbances when treated for 56 days with 20 mg/kg b.w. Carcinogenicity studies in mice and rats revealed no tumorigenic activity. A multigeneration-reproduction study and teratology studies did not suggest any adverse reproductive effects. Mutagenicity tests in prokaryots bacteria were negative, but genotoxic activity was noted in Drosophila and clastogenic activity in bone marrow of mice.

2. Of the substances contained in Pancoxin (*) and Pancoxin Plus (*), sulphaquinoxaline and pyrimethamine have antibacterial properties. Their activity is less than that of sulphamethazol or trimethoprim, however. In Pancoxin Plus (*) the two substances show none of the synergistic effects which have been described in the case of sulphame- thazol and trimethoprim (Guinée 1974, Walter and Heilmeyer 1975).

The effects of sulthaquinoxaline on the percentage of sulphonamide-resistant E. coli in the intestinal flora of chickens were studied in vitro and in vivo (Guinée 1974, Guinée and Kruyt 1975). No significant difference was observed between treated and untreated animals. However, these experiments did not conclusively demonstrate that the use of Pancoxin (*) or Pancoxin Plus (*) in animal nutrition has no effect on the persistance of E. coli multiresistant to drugs since the percentage of these bacteria in the intestinal flora of the experimental animals was very high.

It was found, however, that the resistance to sulphonamides, being located on plasmids, would only exceptionally take the form of monoresistance in E. coli (Renault 1974, Renault and Decourneau 1975). Sulphonamide-resistant E. coli strains show in most cases anything up to a five-fold resistance against antibiotics (Renault and Decourneau 1975, Gedek 1980, 1981, 1982, Siebert 1982, Pohl 1983). The hypothesis that, due to the presence of a quinoxaline cyclic ring, sulphaquinoxaline might have antibacterial properties which are different from those of other sulphonamides has not been demonstrated. No data are available on the question of whether a loss of R-factors may, as in the case of aromatic N-dioxides, result from the administration of sulphaquinoxaline. The available data thus do not allow a reply to question No 2 put by the Commission.

3. Experimental data on the excretion of the individual constituents of Pancoxin (*) and Pancoxin Plus (*) are available for chickens only. Amprolium and ethopabate are eliminated unmetabolized in droppings; sulphaquinoxaline is eliminated for the most part unmetabolized and for a small part as an acetyl derivative while pyrimethamine is excreted as a labile acid conjugate. Amprolium is highly hydrosoluble; sulphaquinoxaline, ethopabate and pyrimethamine are barely soluble. The four compounds showed little toxicity for algae, crustacea and fish, as indicated by the data below :

Chlorella : EC_{50} (mg/l, 2 days) : amprolium : 160, pyrimethamine : 20

Daphnia : EC_{50} (mg/l, 2 days) : amprolium : 230, ethopabate : 170, sulphaquinoxaline : | 7.5, pyrimethamine : 4.8
Lebistes : EC_{50} (mg/l, 2 dyas) : amprolium : 270. LC_{50} (mg/l, 2 days) : ethopabate : 105, sulphaquinoxaline : | 7.5, pyrimethamine : 7.5
Salmo gairdneri : LC_{50} (mg/l, 2 days) : amprolium : 1550, ethopabate : 23, sulphaquinoxaline : | 7.5, pyrimethamine : 5.9.

The phytotoxic potential of Pancoxin Plus (*) has been tested on eight plant species at concentrations between 1 and 1.000 mg/kg soil. No phytotoxicity was observed for lettuce, beans, peas and sunflower. A 50% reduction in plant growth was observed at high concentrations (between 285 and 1067 mg/kg soil) on oats, rape, tomatoes and maize.

In vitro studies showed that Pancoxin (*) as well as Pancoxin Plus (*) do not substantially affect methanogenesis and reduce nitrification only at concentrations distinctly in excess of those that can be attained in soil after spreading of slurry.

The results currently available, although incomplete, suggest that these substances do not constitute a significant environmental risk.

4. In view of the foregoing, the Committee considers that it is unable to issue an opinion on the use of Pancoxin (*) and Pancoxin Plus (*) in feedingstuffs for chickens and turkeys before the following additional data are available :

a) reproduction and teratology studies on amprolium and ethopabate;
b) in vivo mutagenic studies (chromosomal effects) of amprolium, sulphaquinoxaline and ethopabate;
c) metabolism and tissue residues of pyrimethamine in chicken;
d) metabolism and tissue residues of amprolium and ethopabate in turkey;

e) data showing that the metabolism and residues of individual components are not affected when the mixture Pancoxin (*) or Pancoxin Plus (*) is used;

f) effects of Pancoxin (*) and Pancoxin Plus (*) on the persistance of E. coli multiresistant to drugs in the alimentary tract of treated animals;

g) kinetics and degradation of amprolium, sulphaquinoxaline, ethopabate and pyrimethamine in soil and water.

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