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

Opinion expressed 11 December 1980

TERMS OF REFERENCE (March 1980)

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

1. Does the use of the coccidiostat arprinocid in feedingstuffs for chickens, under the proposed conditions of use (see Background), result in the presence of residues in animal products ? Could these residues be harmful to the consumer ?

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

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

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

BACKGROUND

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

Species of animal : chickens for fattening.

Minimum and maximum content in complete feedingstuffs : 60 ppm (mg/kg).

Other provisions : use prohibited at least two days before slaughter.

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

OPINION OF THE COMMITTEE

1. Studies of the metabolism of arprinocid (9-(2-chloro-6-fluorophenylmethyl) 9H-purin-6-amine) in chicken, using the ^{14}C-methylenelabelled compound, show that the compound is rapidly metabolized to the 1-N-oxide, the hypoxanthine, the 2-chloro-6-fluorobenzylalcohol and the 2-chloro-6-fluorobenzoic acid. Only about 0.25 % of administered radioactivity is excreted as $^{14}CO_2$, establishing the metabolic stability of the methylene bridge. Ninety four per cent of the administered radioactivity appears in the mixed excreta; less than 0.1 per cent is found in each of the major organs and in the carcass except for 0.18 % in the liver and 0.37 % in the intestine stripped of its content.

   In surgically altered chicken, permitting separate collection of urine and faeces, 50 % of the administered radioactivity appears in the urine. About one third of the urinary activity is unchanged arprinocid, one third is the 1-N-oxide, one eigth the benzylalcohol derivative and one thirtieth each the hypoxanthine and the benzoic acid derivative. There are considerable species differences in the quantitative but not the qualitative distribution of the urinary metabolites. Residues of arprinocid in tissues and organs appear to consist essentially of 50 % arprinocid, 10 % hypoxanthine and 0.6 % of 1-N-oxide, and the remainder is unidentified metabolites. Residues are found mainly in the liver and kidneys, very little appearing in muscle, skin and fat.

   Residues in tissues and organs of chicken, fed for various times up to several weeks on a ration containing 70 ppm arprinocid, were determined by reverse isotope dilution assays or gas chromatography with electron capture (sensitivity : 50 ppb, limit of detection : 20-30 ppb).

   Three days after withdrawal only about 0.3 ppm residues were detectable in the liver and 0.15 ppm in the kidneys, while none could be detected in other tissues. Five days after withdrawal the liver residues ranged from 0.17 - 0.22 ppm. These liver residues consisted of at least two pools, one representing 85 - 90 % of the residues and consisting essentially of unchanged arprinocid (depletion halflife 1.7 days). The other pool, representing various metabolites, included the 1-N-oxide (depletion halflife 3.5 days). The liver residues appeared to be associated with the macromolecular cellular constituents and were thus largely not bio-available (only 18 %). There were no bio-available residues in liver and kidneys after 5 days withdrawal.

   ^{14}C-labelled arprinocid is well absorbed in rats, 65 % of the radioactivity appearing in the urine, 30 % in the bile and 5 % in the faeces during the 72 hours after administration. When liver containing ^{14}C-labelled arprinocid residues (0.2 ppm after 5 days withdrawal) was fed to rats, most of the activity appeared in the faeces, the net absorption being less than 0.05 ppm.

Arprinocid was investigated in short- and long-term toxicological studies in laboratory animals. The compound possesses hepatic and renal toxicity at high doses as well as having effects on the epithelial and cardiovascular systems manifested by necrosis of ears and tails. It appears to be teratogenic in the rat and mouse but not in the rabbit. This activity is due most likely to the 1-N-oxide metabolite. There was no evidence of carcinogenicity in the two rodent species (rat and mouse) tested nor was there any mutagenic potential demonstrable both in in vivo and in vitro tests. The no-adverse-effect level in the most sensitive species (mouse) was 1 mg/kg body weight. Separate investigation of the 1-N-oxide metabolite revealed no additional toxic potential.

The low bio-availability of the residues in the liver of chicken following relay toxicity tests adds a further safety factor.

2. The absence of activity of arprinocid on various species of micro-organisms tested in vitro (ten bacterial and two fungal strains) indicates that the product has no antimicrobial properties.

3. Studies on the excretion of arprinocid in chicken showed that, under the proposed conditions of use, about 95 % of the amount ingested appears in the excreta within 24 hours after administration. The excreted material is composed of 50 % arprinocid, 4 % 1-N-oxide, about 16 % polar water-soluble metabolites and 7 % bound to faecal solids.

   Aqueous solutions of arprinocid are sensitive to photodegradation, some 70 % being photolysed in 7 days. Tests performed under aerobic and anaerobic conditions showed a slow degradation of arprinocid in undiluted chicken excreta. When excreta were incorporated into different soils, the residues were retained strongly in the soil and little leaching by water occured. The halflife of residues in soil was about 56 days, being slowly degraded to CO_2 and organic volatiles by aerobic processes.

   Arprinocid had low toxicity for algae and fish but was moderately toxic for daphnia. No phytotoxicity was observed in tests carried out in millet, rice, beans, cabbage, cucumber, tomato, carrot and corn crops. These observations indicate that contamination of the environment is unlikely.

4. The Committee is of the opinion, in the light of the available facts, that the use of arprinocid in feedingstuffs for chickens, at a level of 60 ppm (mg/kg), is acceptable provided a withdrawal period of at least 5 days before slaughter is imposed.

REFERENCES

Dossiers Merck and Co Inc.