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

Opinion expressed 10 February 1982

TERMS OF REFERENCE (November 1980)

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

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

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

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

BACKGROUND

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

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

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

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

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

OPINION OF THE COMMITTEE

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

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

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

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

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

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

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

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

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

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

REFERENCES

Dossiers American Cyanamid Co.

Rijksinstituut voor de Volksgezondheid, Bilthoven, Nederland : Internal reports Nos 105/74 and 70/75.