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

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

(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 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 will result 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 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