EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

REPORT
OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION (SCAN)
OF THE EXTENSION OF USE OF DICLAZURIL (E-771)
TO THE FEEDINGSTUFF FOR RABBITS
(adopted on 28 April 2000)

TERMS OF REFERENCE (1998)

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

(1) Does Diclazuril (2,6-dichloro alpha-(4-chlorophenyl)-4-(4,5 dihydro-3,5-dioxo- 1,2,4-triazin-2 (3H)-yl) benzeneacetonitril; E-771) under the conditions proposed for its use as an additive for rabbits (see background) have significant effects on the prevention of coccidiosis in the rabbits?

(2) Is this use safe for the growing rabbits and the pregnant doe?

(3) Could residues of Diclazuril have an adverse effect on the consumer?

(4) Is the use of diclazuril in the diet of rabbits safe for the environment?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC [(1)], the use of Diclazuril (E-771) is authorised at Community level in the Annex I, Section D (Coccidiostats and other medicinal substances), according to the conditions set up by Commission Directive 93/107/EEC[(2)] as follows (See Table I)

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(1) Concerning additives in feeding stuffs (O.J. N° L 270, 14.12.70, p 1. as amended by Directives 84/58/EEC (O.J. N° . L 319 8/12/84 p 13) and 93/114 EC (O.J. N° L 334 31.12. 93, p.24)

(2) O.J. N° L 219, 04.12.93, p.44

Table I. Annex I, Part D (Coccidiostats). Diclazuril (E-771)

Species of category of animal	Maximum age	Minimum content	Maximum content	Other provisions
		mg/kg of complete feedingstuffs		
Chickens for fattening	-	1		Use prohibited at least five days before slaughter
Turkeys	12 weeks	1	1	Use prohibited at least five days before slaughter
Chickens reared for laying	16 weeks	1	1	-

The Scientific Committee for Animal Nutrition has expressed its favourable opinion in its reports:

- of 10 July 1991 on the use of Diclazuril in feedingstuffs for chickens [3],
- of 24 October 1997 on the use of Diclazuril in feedingstuffs for turkeys, [3]
- of 5 November 1997 on the use of Diclazuril in feedingstuffs for chickens reared for laying [3

An extension of the use of Diclazuril (E-771) to rabbits has been requested under the following conditions of use (see Table II):

Table II. Annex I, Part D (Coccidiostats) Diclazuril

Species of category of animal	Maximum age weeks	Minimum content	Maximum content	Other provisions
		mg/kg of complete feedingstuffs		
Rabbits		1	1	Use prohibited at least 1 day before slaughter

A registration file has been submitted by the applicant firm divided into four sections describing:

I - Summary highlights

II - Identity characterisation, methods of control

III- Efficiency of the additive

IV - Safety of the additive

[3] See Series of reports SCAN Reports in Section References

OPINION OF THE COMMITTEE

1. EFFICACY

Laboratory studies, comparative laboratory trials, field trial and studies concerning the quality of animal product have been performed to demonstrate the efficacy of Diclazuril as a coccidiostat in rabbits. Seventeen experiments have been carried out in six countries of Europe.

Eight laboratory trials (all including experimental inoculation of different species of *Eimeria)*; four comparative laboratory trials (two of them including experimental inoculation) and five field trials (conducted without inoculation) were performed: seven in Belgium, three in Spain, three in Italy, two in France, one in Germany and one in Yugoslavia.

1.1. Studies design

Ten experiments were performed to study the efficacy of Diclazuril using dose-response titration from 0.1 to 3 mg of the additive / kg of complete feedingstuff, following experimental inoculation of the animals with sporulated oocysts of *Eimeria*. The following *Eimeria* strains were the most commonly found in rabbits: *E. magna, E. intestinalis, E. perforans, E. stiediae, E. flavescens, E. irresidua, E. media* and *E piriformis.* The inoculation was carried out on around 4-7 weeks aged rabbits, receiving 50,000 to 150,000 units/head of either a single species or a combination of the following four species: *E. intestinalis, E. magna, E. perforans* and *E. irresidual*. Animals were raised in wire floored cages either individual, or in groups of two to eight animals.

Diclazuril efficacy was evaluated using a common experimental design with three categories of growing rabbits: uninfected untreated controls (UUC), infected untreated controls (IUC) and infected treated animals (ITA), fed different levels of Diclazuril in their diets under treatments lasting 5 - 56 days. The majority of the treatments lasted 21 days. Faecal oocysts counts and zootechnical parameters (weight gain, feed consumption and feed conversion ratio) were recorded during the week following the inoculation or, treatment in five experiments, over the whole period of treatment. In the remaining five experiments, faecal oocysts score, the same zootechnical parameters together with mortality rate, liver weight and liver lesions scores were also recorded.

In one additional comparative laboratory trial the effect of treatment with 1 and 2 mg Diclazuril/kg of complete feedingstuff on the performance of fattening rabbits was tested.

Data collected in comparative laboratory studies, field studies considered the effect of treatment with 1 mg Diclazuril / kg of complete feedingstuff on the reproductive performance of does, not previously experimentally inoculated.

1.2. Results of the studies

The conclusions from the studies carried out by the Company were:

Experimental infection by *E. magna* or *E. flavescens* proved to be symptomatically more severe than those by *E. intestinalis* or *E. irresidua*, the former requiring a higher level of Diclazuril to control coccidiosis. The absence of oocysts in faeces over one week of treatment both for animals infected with a single species or the four previously quoted species showed that treatments with 1, 2 or 3 mg Diclazuril/kg of complete feedingstuffs were equivalent in terms of coccidiosis control. The faecal score improved significantly after 21 days of treatment. The treatment reduced mortality rate, increased weight gain and improved feed efficiency.

Comparative laboratory (four experiments) and field studies (five experiments) were performed on 350 and 10,945 rabbits respectively, including 380 reproductive does. The studies compared the efficacy of the inclusion of 1 mg Diclazuril/kg of complete feed for fattening rabbits or reproductive does, raised on a wired floor to prevent recontamination. The faecal excretion of oocysts in treated animals compared with that in untreated ones decreased down to zero and the score of lesions and diarrhoea improved remarkably. Mortality rate was reduced and liver lesions disappeared almost completely in treated animals. Even after a nine week interruption of treatment, no adverse effect on zootechnical parameters i.e. feed efficiency and weight gain were observed in adult animals raised in commercial breedings. Thus, at the level of 1 mg additive/kg of complete feed, Diclazuril was effective for the control of coccidiosis from *E.magna, E. media, E. perforans, E. intestinalis, E. irresidua* and *E. stiedae.*

Diclazuril efficacy at levels from 0,5 to 2,0 mg/complete feed was compared with that of two other anticoccidial products used at their proper levels as additives. Diclazuril at 1 mg/kg complete feed always proved more effective than these compounds in controlling coccidiosis both in fattening rabbits and in reproductive does. Mortality rate and hepatic damage were reduced in animals on the Diclazuril diet compared to controls. Weight gain and feed conversion index improved. Performances and feed efficiency were improved also in does, even following the discontinuation of the treatment performed in commercial breedings.

Conclusion

Diclazuril included in the diet of growing rabbits and reproductive does at the level of 1 mg/kg of complete feedingstuff is an effective anticoccidial additive for the control of *Eimeria* infections.

2. TOLERANCE AND REPRODUCTIVE TOXICITY

2.1. Tolerance tests in target animals

Tolerance of Diclazuril was first verified with weaned healthy rabbits of both sexes. Three consecutive experiments were performed, using for each assay, 60 rabbits from ten litters and fed for 5 consecutive weeks with diets containing the following levels of Diclazuril: 0; 0.38; 1.93; 7.31; 28.9 and 119.6 mg/kg of complete feed in the diet, (obtained from the complete feed analyses).

A very high mortality rate of animals was observed during the experiment, particularly between the third and the fifth week, in all groups including the control. Out of 180 rabbits, only 43 animals (14 males and 29 females) survived at the end of the experiment. No macroscopic and microscopic examination were performed on animals that died during the experiment.

The company was asked for clarification on 23 November 1998. The answer received in May 1999 did not supply additional information.

Conclusion

As a consequence of the high rate of mortality and the low and imbalanced number of surviving animals between treatments and between sexes, no conclusion on the tolerance test in rabbits can be reached.

2.2. Embryotoxicity and teratogenicity

Reproductive toxicity studies in Diclazuril have been carried out on 100 days aged females. The animals were fed 0, 1 or 6 mg Diclazuril/kg of complete feedingstuff, respectively continuously for ten months during their reproductive cycle. Data collected included the age of the does at first farrowing, the weight at ten days following parturition, the causes of culling, the number of matings, the litter size expressed as the number of new-born per litter, their viability and the litter size at weaning and growth performance of young rabbits. Data were issued from 544 gestations of females completing at least three successive litters at weaning. No significant effects of treatments were found for all parameters recorded. In addition, according to results of experiments conducted for efficacy, continuous feeding of reproductive does with Diclazuril at the proposed level of 1 mg/kg complete feedingstuff did not affect their fertility and prolificity as well as the litter size and the viability of the new born rabbits.

Reproductive toxicity on the male has not been tested. However data from field trials collected on 380 females rabbits treated at the recommended level and naturally mated by males treated in the same way did not show any depressive effect on their reproductive performance.

Embryotoxicity and teratogenicity were firstly examined on four groups of 15 does submitted to artificial insemination. Each were administered Diclazuril parenterally or orally by gavage from day 6 to 18 of pregnancy at doses of: 0 ; 5 ; 20 or 80 mg Diclazuril/kg bw. No information was given on the

treatment of males providing the semen for insemination. Individual daily records mentioned signs of discomfort or abnormal behaviour. None of the parameters concerning pregnancy, body weight and mortality, litter size, foetus weight, number of resorptions, dead and live foetuses and abnormalities at 28 days of gestation observed after autopsy, were adversely affected even at the maximum level of treatment. The gross necropsy parameters on all progeny, including the examination of legs, tail, ribs, bones and intestines, failed to demonstrate teratogenic effects or abnormalities in foetuses at any level of treatment.

A second experiment on embryotoxicity and teratogenicity used four groups of 15 adult females (3-4kg) administered 0; 40; 80; or 160 mg Diclazuril /kg bw daily by gavage from day 6 to day 18 of pregnancy. No effect on mortality rate and reproductive performance of the mother and the foetuses were noted. Radiographic examinations of all of the foetuses at 28 days of pregnancy showed no major malformations and no drug or dose-related effect on the progeny was detected.

Conclusion

Data on reproductive performance of does and on embryotoxicity conducted at the highest level of dosage, *i.e.* 6 mg Diclazuril/kg of complete feedingstuff and 160 mg Diclazuril/kg bw, respectively demonstrated that no deleterious effect of the product was observed in the female rabbit treated during pregnancy and in the offspring.

3. METABOLISM AND RESIDUES

3.1. Metabolic fate

A radiometric study has been performed in adult New Zealand white rabbits using a single oral dose of 1 mg /kg bw of ^{14}C-Diclazuril labelled on the nitriles–group.

Plasma radioactivity levels of unchanged Diclazuril reach a maximum level of 0.79-1.4 µg-eq./ml within 6 hours after dosing that persisted until 48 hours. From 48 hours after dosing, this concentration decreased somewhat irregularly, apparently following a biphasic decline. (half-life of 2-2.5 days for the second phase).

Excretion of radioactivity was fairly rapid, since 73% of the dose was already recovered in the excreta at 48 hours after dosing (70% in the faeces and 3% in the urine). Ten days after dosing, more than 98% of the radioactivity was excreted. Data on the distribution of the radioactivity in different parts of the digestive tract showed that six hours after dosing 1.3 to 9.6% of the administrated dose was present in the content of the stomach, whereas 0.6-3.6% was present in the contents of the colon, 14-34% in the caecum and 1.6-7.2% in the rectum. At that time a constant level of radioactivity within a range of 0.24-0.32 µg/g occurred in the tissues of various parts of the intestinal tract. If these data would indicate the rapid passage of Diclazuril through the intestinal tract, the sustained peak concentration in plasma and

most tissues for at least 48 hours is consistent with a delayed absorption of Diclazuril, which might be associated with caecotrophy and subsequent recycling of a fraction of the administered dose and a low absorption. However, no data was available concerning the biliary excretion to assess the extent of the absorption of the compound.

The distribution of the radioactivity into tissues following a single dose administration was limited and the elimination proceeded with the same elimination half-life as that in the plasma.

In plasma, unchanged Diclazuril accounted for almost the complete sample radioactivity at least up to 120 hours after dosing. Data available on the biliary excretion after 6, 48, 120 and 240 hours following the administration of a simple dose of Diclazuril demonstrated that the maximum level of total Diclazuril is < 0.02 µg/g and consequently that the biliary excretion is negligible. In urine, unchanged Diclazuril represented only 6.2% of the total radioactivity and 5 additional different metabolites were detected, particularly a glucuronide and a sulphate conjugate of the same hydroxylated metabolite which represented 25 and 15 % of the 0-120 hours total radioactivity, respectively. In faeces, there was an extensive faecal excretion of the unchanged substance accounting for 43-69% of the dose in 24 hours after dosing. No information is available concerning the nature of residues in tissues. The only data concerned bound residues in the liver which represented only 6% of the total radioactivity.

3.2. Tissue residues

In a preliminary study, groups of eight (four males and four females) young adult rabbits, eight weeks of age, were dosed for 14 days with Diclazuril, admixed at 1 ppm in the feed. Plasma and edible tissues were sampled at 1 and 7-day time points after the last dose and the concentration of Diclazuril measured. Steady-state was attained within ten days continuous feeding. The average concentrations of Diclazuril in liver, kidney and fat were 1.59 µg/g. 0.64 µg/g and about 0.20µg/g one day after the last dose. A concentration of 0.71 µg/g was found in the liver after seven-day withdrawal, whereas in kidney and fat, levels were undetectable. However, there are no data on the identification of the residues in the liver at 6, 24, 72, 120, 168 and 240 h, respectively after a multidosing and in turkey after a single dose (SCAN, 1991 and 1998). Despite unchanged Diclazuril accounted for the major part of liver residues during 24 h after dosing, it is clear that its metabolites are present in significant amounts during the following days. No residues of Diclazuril could be detected in muscle even after a one-day withdrawal period. There was no evidence for sex-related differences with respect to plasma levels and tissue residues of Diclazuril.

In a second study, the residue concentrations were evaluated 1, 3, 5 and 10 days after the last dose and samples of liver, kidney, muscle and fat were collected individually from eight animals of both sexes (four animals per sex). Tissue samples were analysed for Diclazuril using a HPLC validated method with a detection limit of 0.050 µg/g in kidney, liver and muscle and 0.10 µg/g

in fat. Highest tissue levels were observed in liver (i.e. target tissue) and lowest in muscle and fat.

Conclusion

The data on absorption, tissue distribution, excretion and metabolism indicate that Diclazuril is metabolised to a limited extent and that the distribution of Diclazuril and metabolites in the tissues is limited. However no confirmation is given that unchanged Diclazuril is the marker in the target tissue.

4. SAFETY ASSESSMENT FOR THE HUMAN CONSUMER : ADI PROPOSAL FOR DICLAZURIL AND WITHDRAWAL PERIOD PROPOSAL FOR DICLAZURIL IN RABBITS

A toxicological ADI of 0.029 mg/kg bw/day (equivalent to 1.74 mg for a 60 kg person) could be proposed for Diclazuril by applying a safety factor of 100 to the lowest NOEL of 16 mg Diclazuril /kg of complete feedingstuff. This is equivalent to approximately 2.9 mg/kg bw established from the carcinogenicity male mouse study (SCAN, 1991, page 5 of the report).

It is noted that Diclazuril is a racemate (a 50/50 mix of isomers). No information is available on the relative toxicities of the isomers nor on the ratio of the isomers of Diclazuril present in food as residues. As a worst-case approach, it is assumed that all of the toxicity of Diclazuril was due to one isomer that makes up the whole of the food residue and thus the theoretical maximum intake of Diclazuril should not take up more than 50% of the ADI.

The theoretical maximum daily intake of Diclazuril based on residues levels at 1, 3, 5, 7 and 10 day withdrawal period after treatment of rabbits receiving 1 mg Diclazuril /kg of complete feedingstuff for 14 days has been estimated to 188.0, 114.8, 96.4, 75.3 and 49.5 µg per day respectively. However, as it was not confirmed that Diclazuril is the marker residue, this calculation cannot apply.

Conclusion

In spite of a toxicological evaluation that allows to set an ADI, in the absence of data on the residues in the target tissue to establish the marker residue, it is not possible for the SCAN to conclude that a withdrawal period of one day is adequate to protect the consumer.

5. ENVIRONMENTAL IMPACT OF EXCRETED DICLAZURIL

5.1. Soil

Depending on regulations on the input of fertiliser with manure and whether it is incorporated into the soil, the Predicted Environmental Concentrations (PEC) in soil can vary from 1.1 up to 14 µg/kg.

In addition, Diclazuril is very strongly adsorbed onto soil and, at concentrations in the range from 10 to 100 µg/kg, the residues disappeared from soil with an elimination half-life of less than 60 days.

Therefore, when used at the recommended dose, resulting concentrations in the soil will not have detectable effect on terrestrial animals and higher plants (SCAN, 1991).

5.2. Aquatic environment

- Algae are not affected at levels up to 0.15 mg/l and growth reduction is seen only with concentrations over 4.8 mg/l.

- Diclazuril levels higher than 0.5 mg/l are required to affect the growth of the aquatic invertebrate *Daphnia magna*.

- The LC_{50} for fish (*Lepomis macrochirus*) is 0.58 mg/l while the observed Lowest Observed Effect Concentration (LOEC) was 0.26 mg/l.

The above data show that the toxicity of Diclazuril to aquatic organism occurs only at very high concentrations.

In addition, Diclazuril is very poorly soluble in water and can only be extracted by organic solvents, generally not present in the environment.

Finally, PEC in water leaching from the soil was in the worst case of exposure to rabbit manure, about 5,000 times lower than the LOEC.

5.3. Conclusion

The strong adsorption of Diclazuril onto soil, its short half-life in soils and its poor water solubility lead to the conclusion that no detectable effect could be expected on terrestrial or aquatic organisms.

6. CONCLUSIONS

6.1. Diclazuril included in the diet of growing rabbits and reproductive does at the level of 1 mg/kg of complete feedingstuff is an effective anticoccidial additive for the control of *Eimeria* infections.

6.2. The poor quality of the tolerance study submitted did not allow the SCAN to conclude on the safety margin of Diclazuril for the target animal.

Data on the effect of the treatment of pregnant does are satisfactory. However the absence of treatment of males does not allow SCAN to fully assess the impact of the use of Diclazuril on reproductive performance.

Therefore the safety of use of Diclazuril in the target animal categories is not demonstrated.

6.3. In the absence of data concerning the nature of Diclazuril residues in tissues, it is not possible for the SCAN to conclude on the safety of Diclazuril for the human consumer.

6.4. Because of the PEC values in soil and water, the use of Diclazuril is safe for the environment.

On the basis of these conclusions, the SCAN does not recommend the use of Diclazuril as feed additive for rabbits.

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