European Medicines Agency
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

DICLAZURIL

SUMMARY REPORT (1)

1. Diclazuril, a benzeneacetonitrile derivative is an anticoccidial intended for oral use in lambs. The therapeutic dosage is either a single administration of 1 mg diclazuril per kgbw (most commonly at about 6-8 weeks of age) or two administrations (the first time at 3 to 4 weeks of age and the second time about 3 weeks later).

   Diclazuril has been registered as an anticoccidial feed additive for broilers with a withdrawal period of 5 days in the Council Directive 93/107/EC of 26 November 93 amending Council Directive 70/524/EEC.

2. Although the mode of action of diclazuril is not precisely known, its effect on the asexual or sexual stages of coccidies blocks the excretion of oocysts inducing an interruption of the life cycle of the parasites.

3. Four pharmacokinetic studies were conducting in rats using ^{14}C-diclazuril. After oral administration of 10 mg of diclazuril/kgbw, the maximum plasma level, 1 mg equivalent/l, occurred 8 hours post dosing. The area under the curve (AUC) estimated for the unchanged drug represented about 75% of the total radioactivity AUC .

   At 24 hours post dosing, 0.2% of the radioactivity was recovered in urine and nearly 90% in the faeces the unchanged drug accounting for 86-89%. Four minor metabolites were detected in the faeces extracts, each representing less than 1% of the radioactivity.

4. At the end of the three-month oral toxicity study carried out in rats (100, 200 and 300 mg/kgbw/day) and in mice (200, 400 and 600 mg/kgbw/day), the plasma assays showed that diclazuril concentrations were not dose-dependent for the highest dosage. This loss of linearity might be due to a phenomena of the saturation of diclazuril absorption at high dose levels.

5. Plasma pharmacokinetic studies were carried out in chickens and turkeys with ^{14}C -diclazuril and in rabbits and sheep without radiolabelled compound.

   In birds, the highest concentration of radioactivity, about 1.80 mg equivalent/l, was observed at 6 h after a single oral administration of 1 mg ^{14}C -diclazuril/kg. Plasma radioactivity was due almost exclusively to unchanged diclazuril for up to 72 h after dosing.

   In rabbits, the diclazuril plasma concentrations were lower than 1 mg/l during the 14-day treatment by diclazuril admixed in feed at a level dose of 1 ppm (equivalent to 0.067 mg/kgbw/day). After the end of the treatment, they decreased slowly to attain 0.2 mg diclazuril/l 7 days later.

   In lambs after two oral administrations of 1 mg/kgbw of diclazuril separated by 3 weeks, the maximum plasma levels amounted to 0.150 mg/l and to 0.080 mg/l, 24 hours after the first and second doses respectively showing that oral availibility is low.

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6. After a single oral administration of 1 mg/kg bw of ^{14}C-diclazuril to rabbits, chickens or turkeys, 70% of the radioactivity were excreted in faeces within 24 hours for rabbits and 50% for chicken and turkeys. More than 98% were recovered within 10 days. In faeces the parent compound represented 85 to 95% of the radioactivity excreted. Each of the several minor faecal metabolites accounted for less than 1% of the radioactivity excreted in general. As an exception, a derivative of 4-amino-2,6-dichloro-α-(4-chlorophenyl)benzeneacetonitrile, detected in birds accounted for 8%. In rabbits, 3% of the radioactivity was recovered in urine. Among the six urinary metabolites detected, a glucuronide and a sulphate compound were identified.

7. An *in vitro* comparison of the metabolism of diclazuril was carried out in primary hepatocyte cultures of sheep, chickens, turkeys, rats, rabbits and goats. The pharmacokinetic profile and metabolism of diclazuril was similar in all these species. The parent compound represented more than 95 % of the radioactivity for chickens and sheep, about 85 % for rabbits and rats, 65.1 % for turkeys and 59.7 % for goats, in which the metabolism was most extensive.

8. In acute toxicity studies, oral or subcutaneous dosages up to 5000 mg/kgbw caused no mortality in mice and rats. However, the intraperitoneal LD_{50} in male rats was 5000 mg/kgbw diclazuril was well tolerated up to 2240 mg/m^3 by inhalation in rats and up to 4000 mg/kgbw after dermal application to rabbits.

9. Sub-chronic toxicity of diclazuril was well studied in mice, rats and dogs.

   In two three-month toxicity studies, mice received diclazuril in the diet at dosage levels ranging from 200 to 3000 ppm. Swelling of the centrilobular hepatocytes was reported for doses higher than 400 ppm for males and in the 1600 ppm dosed females. No toxic effects were reported for the dose level of 200 ppm (approximately 50 mg/kgbw/day) for males and 800 ppm (equivalent to 200 mg/kgbw) for females.

   In rats, diclazuril was administered in the diet, for 3 months, at level doses ranging from 50 to 3000 ppm. In the two highest dose-groups (2000 and 3000 ppm), hepatocytic cytoplasmic vacuolisation and an increased incidence of eosinophilic cytoplasmic condensates were reported. Histopathological examination showed swelling of the centrilobular hepatocytes in both males and females. A NOEL of 50 ppm (4.38 mg/kg bw/day) for males and of 200 ppm (20.8 mg/kg bw/day) for females were retained, based on the absence of hepatic lesions.

   In dogs, diclazuril was administered in gelatine capsules at 5, 20 and 80 mg/kg bw/day for 3 months. At the top dose, in both sex, a significant increase of fine granular yellowish to brown pigments in the cytoplasm of the hepatocytes was observed. A NOEL of 20 mg/kg bw/day was retained based on the absence of hepatic lesions. After a 1 month recovery period, the above variations and liver changes were no longer seen, indicating they were reversible.

10. Two twelve-month toxicity studies were performed in rats and dogs.

    In rats, diclazuril was given via feed at dose levels of 16, 63, 250 and 1000 ppm. In addition to the findings previously reported, histiocytic aggregates in the mesenteric lymph nodes and foamy cells in the lungs were observed in females which leads to a NOEL of 63 ppm (5.76 mg/kgbw/day) for females.

    In dogs, after oral administration of diclazuril at 5, 20 and 80 mg/kg bw/day for 12 months, the toxic effects were similar to those reported for the 3-month toxicity study and the NOEL was 20 mg/kgbw/day.

11. In tolerance studies, no drug-related abnormal clinical observations nor side-effects were observed in sheep after treatment at 1, 3 or 5 times the proposed therapeutic dose.

12. A two-generation reproduction study with 2 litters per generation in rats was performed, diclazuril being administered in feed at dose levels equivalent to 5, 20 and 80 mg/kgbw/day. Maternotoxicity, characterised by a decrease of bodyweight gain and of food consumption, appeared for doses higher than 20 mg/kgbw. Foetotoxic effects, decrease in pups weight at weaning and decrease in survival rates at 3 weeks of age, were reported for the two highest dosages. No teratogenic effects were recorded. 5 mg/kgbw/day can be considered as the NOEL.

13. Teratogenicity studies were carried out in rats and rabbits.

    In rats, diclazuril was given in the feed at doses equivalent ranging from 1.25 to 160 mg/kgbw No adverse effects on dams and pups were observed for dosages up to 5 mg/kgbw. No teratogenicity was observed. 5 mg/kgbw/day is the NOEL level.

    In two studies conducted in rabbits, diclazuril was administered by gavage in rabbits at doses between 5 to 160 mg/kg/day. No adverse effects were seen on dams or litters.

14. The mutagenic potential of diclazuril was explored in five *in vitro* tests (Ames test, L5178Y/TK Mouse Lymphoma assay, lymphocytes chromosome aberration test, UDS test on rat hepatocytes in primary culture, SOS chromotest in *E.coli*) and in three *in vivo* tests (sex-linked recessive lethal test in drosophila, micronucleus test in mice, dominant lethal in male mice). The negative results obtained enabled to conclude that diclazuril was not genotoxic.

15. A 25-month chronic/carcinogenicity study was conducted in Charles River Swiss mice. Diclazuril was administered in the diet at level doses of 16, 63, 250 and 1000 ppm. The same adverse effects as those described in the subchronic studies were recorded. The NOELs were 63 ppm (14.1 mg/kg bw/day) for females and 16 ppm (2.9 mg/kg bw/day) for males. No carcinogenic potential could be seen.

16. In a 28-month chronic/carcinogenicity study, the Wistar rats received 0, 16, 63, 250 and 1000 ppm of diclazuril. Histiocytosis of the mesenteric lymph nodes were described in males treated at 1000 ppm and in females at the two top doses. 63 ppm (5 mg/kg bw/day) in females was the highest NOEL for chronic dosing in the most sensitive sex of the rat species. No carcinogenic properties of diclazuril could be seen.

17. Diclazuril did not possess any antifungal activity and was devoid of activity at 100 µg/ml against *Bacillus subtilis* and *Sarcina lutea*.

18. This compound has already been assessed at the 45th JECFA-meeting. A temporary rounded ADI of 0.020 mg/kgbw was established based on a NOEL of 3 mg/kgbw/day from the chronic/carcinogenicity study in mice and applying of a factor of 200, due to the absence of marked maternotoxicity in the rabbit teratogenicity study.

19. Based on the same value considered by JECFA and retaining a safety factor of 100, a toxicological ADI of 0.030 mg/kg/day can be established (i.e. 1.80 mg/day per 60 kgbw person).

20. Two radiometric depletion studies were carried out in broilers.

    Twenty-four hours after a single administration of 1 mg of ^{14}C-diclazuril per kgbw, 0.10 mg equivalent diclazuril/kg were measured in fat and muscle, 0.92 mg equivalent diclazuril/kg in liver and 0.72 mg equivalent diclazuril/kg in kidney. The radioactivity decreased to reach, 72 hours after dosing, 0.05 mg equivalent diclazuril/kg in fat and muscle, 0.42 mg equivalent diclazuril/kg in liver and kidney.

    Twenty-four hours after a repeated administration of 0.090 mg of diclazuril/kgbw/day for 14 days, 0.04 mg equivalent diclazuril/kg were detected in muscle, 0.11 mg equivalent diclazuril/kg in fat and 0.24 mg equivalent diclazuril/kg in liver respectively. These levels decreased to reach approximately 0.02, 0.80, 0.20 mg equivalent diclazuril/kg in muscle, fat and liver, 72 hours post dosing.

    The average half-life for the depletion of radioactivity from tissues ranged from 71 hours (liver) to 60 hours in the muscle.

    In 24 hour-liver samples, more than 95% of the radioactivity could be extracted. At 24 hours post dosing the ratio of parent compound to total residues was 85.90% in muscle, 77.27% in fat and 84.20% in liver.

21. Two radiometric depletion studies were carried out in turkeys.

    Forty-eight hours after a single administration of 1 mg of ^{14}C-diclazuril per kgbw, the total radioactivity levels were 0.08 mg equivalent diclazuril/kg in muscle, 0.21 mg equivalent diclazuril/kg in fat, 0.71 mg equivalent diclazuril/kg in liver and 0.45 mg equivalent diclazuril/kg in kidney. In liver, 100% of the radioactivity could be extracted. The ratio of parent compound to total residues was 83% in muscle, 55% in fat, 77% in liver and 97% in kidney.

    Six hours after the cessation of a 14-day administration of 0.050 mg of diclazuril/kgbw/day for 14 days, the residues in muscle, fat, liver and kidney were 0.065, 0.247, 0.509, 0.371 mg equivalent diclazuril/kg.

22. One radiometric depletion study was carried out in rabbits.

    Forty-eight hours after a single administration of 1 mg of ^{14}C-diclazuril per kgbw, radioactivity levels were measured in liver (2 mg equivalent diclazuril/kg), kidney (1.1 mg equivalent diclazuril/kg) and fat (0.03 mg equivalent diclazuril/kg). In muscle, the concentrations did not exceed 0.01 mg equivalent diclazuril/kg. The radioactivity elimination half-life was 2-2.5 days for all tissues, except for the liver (3 days).

23. In two non-radiometric depletion studies carried out in rabbits after administration of diclazuril in feed at doses equivalent to 0.067 mg/kgbw/day for 14 days, the concentrations of diclazuril in tissues at 24 h post-dosing, were 0.20 mg/kg in fat, 1.60 mg/kg in liver, 0.60 mg/kg in kidney. No residue could be detected in muscle tissue.

24. In sheep, two non-radiometric depletion studies were carried out.

    Twenty-four hours after a single administration of 1 mg/kgbw to 3-week-old lambs, 0.028 mg of diclazuril/kg were measured in muscle, 0.084 mg/kg in fat, 0.298 mg/kg in liver and 0.092 mg/kg in kidney.

    In a second study, twenty four hours after administration of 1 mg/kgbw to 7 week-old lambs, the following concentrations of diclazuril were measured in tissues : 0.013 mg/kg in muscle, 0.042 mg/kg in fat, 0.280 mg/kg in liver and 0.038 mg/kg in kidney. Residues of diclazuril were below the limit of quantification 0.010 mg/kg at day 3 for muscle, day 5 for kidney and fat and day 7 for liver.

25. A gas chromatographic method with an electron capture detector has been developed to measure residues of diclazuril in sheep edible tissues and the limit of quantification was 0.010 mg/kg.

Conclusions and recommendation

Having considering the criteria laid down by the Committee for Veterinary Medicinal Products for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that :

- diclazuril is of low toxicity;
- the animals are unlikely to be sent for slaughter immediately after treatment;
- at 24 hours post-treatment, the consumer intake represents only 2.75 % of the toxicological ADI of 0.030 mg/kg bw,

the Committee considers that there is no need to establish an MRL for diclazuril and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacalogically active substance(s)	Animal species	Other provisions
Diclazuril	Ovine	For oral use in lambs only