The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

CLAZURIL

SUMMARY REPORT

1. Clazuril, (±)-2-chloro-a-(4-chlorophenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin)-2(3H)-yl)-benzeneacetonitrile, is an anticoccidial intended for oral use in pigeons. The recommended dosage is one tablet containing 2.5 mg clazuril per pigeon per month.

2. Although the mode of action of clazuril is not precisely known, a lethal effect on *Eimeria* species was seen in histological examinations.

3. The plasma kinetics were studied in the rat which received daily oral doses of 5, 20, or 80 mg/kg bw. The average clazuril concentration in plasma, sampled 2 hours after dosing on days 2, 5, 10, 17 and 29, were 15.2 ± 4.0 µg/ml (n=10) after doses of 5 mg/kg bw; 43.4 ± 8.1 µg/ml after doses of 20 mg/kg bw; and 71.2 ± 13.3 µg/ml after doses of 80 mg/kg bw for the male rats, and 4.67 ± 2.14 µg/ml, 14.4 ± 3.3 µg/ml and 56.6 ± 5.8 µg/ml, respectively, for the same dosage in female rats. The plasma concentrations of clazuril were higher in male than in female rats.

4. In a single-dose toxicity study in rat, no mortalities occurred in males or females at an oral dosage of 640 mg/kg bw. Bodyweight was not affected. Behavioural effects were of central nervous system-nature. Pathological examination revealed no drug-specific changes. In pigeons, dosing up to 640 mg/kg bw remained without mortality. The only side effects observed were vomiting and transiently soft faeces at 80, 320 and 640 mg/kg bw.

5. The repeated-dose toxicity was studied in rats and in pigeons following daily oral administration for 1 month.

   In rats dose levels were 0, 5, 20 and 80 mg/kg bw (n=5). Some slight alterations on serum parameters at 20 mg/kg bw were not associated with pathological changes. Dosing at 80 mg/kg bw resulted in a slightly decreased bodyweight gain. Blood analysis indicated an increase in the number of segmented neutrophils and a decrease in the number of lymphocytes. Serum analysis revealed a decrease in triglycerides and potassium and an increase in blood urea nitrogen and creatinine in males. In females, a decrease in triglycerides, total bilirubin and aspartate aminotransferase and an increase in blood urea nitrogen and cholesterol were found. Centrilobular swelling of the hepatocytes and a slightly increased testicular degeneration were observed histologically. The NOEL was 5 mg/kg bw.

   Pigeons were given 0, 5, 10 and 15 mg/kg bw (n=8). At 10 mg/kg bw, a slightly increased liver weight was noted, histologically confirmed by an increase in hepatocellular vacuoles. Dosing at 15 mg/kg bw resulted in a marked body weight loss and histological liver changes being an increase in hepatocellular vacuoles and the presence of bile plugs. The NOEL was 5 mg/kg bw.

6. In a tolerance study with adult pigeons (n=121) oral doses of 0.63, 1.25, 2.5, 5 and 10 mg/pigeon did not cause any side-effect.

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A weekly oral dose of 2.5 mg/pigeon of clazuril was given to young, unweaned pigeons (n=28, and n=15 in a placebo group). One pigeon died at the age of 10 days in the control group. Six pigeons died in the treated group, for three it was due to nest fighting while for the three others the ethiology of death could not be determined. Bodyweight gain was comparable between control and treated animals.

7. The effect of clazuril on reproductive performance was investigated in pigeons (n=128). The first oral treatment (0, 5 and 10 mg/kg bw) was administered before coupling and further at weekly intervals for 6 months. Within each dosage group, 4 experimental groups were formed: treated males-treated females, treated males-untreated females, untreated males-treated females and untreated males-untreated females. The measured parameters were reproductive behaviour, egg laying, fertilized/unfertilized eggs, embryonic death, number of hatched chicks, number of weaned chicks, maternal and paternal bodyweight, feathering, interval between successive reproduction cycles, gross pathology and for the new-born pigeons: mortality, bodyweight gain, age of weaning, feathering, X-ray to detect skeletal malformations and autopsy. No maternal or paternal toxicity was evident. Reproductive behaviour was comparable in the various groups. In the offspring, survival, clinical behaviour, feathering and body weight gain were unaffected. Gross pathological examination revealed no adverse effects.

8. Embryotoxic and foetotoxic effects were investigated in a segment II study where clazuril was given orally at dose levels of 0, 1.25, 5 and 20 mg/kg bw to female rats (n=96) from day 6 post conception to day 16. The study revealed no maternal, embryotoxic or teratogenic effects up to 5 mg/kg bw. At the dose of 20 mg/kg bw (4 times the recommended therapeutic dose), a slight increase of embryonal resorption was observed and the bodyweight of the pups were slightly decreased. From this study a NOEL of 5 mg/kg bw can be established.

9. Clazuril was studied for its mutagenic potential *in vitro* and *in vivo*. The DNA-damaging potential was evaluated by a SOS-chromotest in *Escherichia coli*. The potential for inducing point/gene mutations was assessed *in vitro* by the Ames test in *Salmonella typhimurium* (TA 1538, TA 98, TA 97, TA 1535 and TA 100) and the potential for induction of chromosome aberrations was evaluated *in vivo* by the micronucleus test in male and female mice (40, 160 and 640 mg/kg bw). No mutagenic potential was demonstrated in any of these studies.

10. Based on a NOEL of 5 mg/kg bw/day from the repeated dose toxicity study in rats and pigeons and applying a safety factor of 100, a toxicological ADI of 0.05 mg/kg bw can be established (i.e. 3 mg/person).

11. No pharmacokinetic and radiometric studies in the target species were provided. The pharmacokinetic behaviour of clazuril was extrapolated from studies performed on broiler chicken and on turkey with its dichloro-analogue: diclazuril ((±)-2,6-dichloro-a-(4-chloro-phenyl)-4-(4,5-dihydro-3,5-dioxo-1,2,4-triazin-2(3H)-yl)benzeneacetonitrile). The structural analogy between diclazuril and clazuril allows the extrapolation.

    Male and female broiler chickens (n=36) were given a single oral dose of ^{14}C-diclazuril (1 mg/kg bw). Up to 240 hours after dosing, samples of plasma, tissues and excreta were collected to allow the quantification and characterisation of the residual diclazuril-related radioactivity. Peak plasma concentrations of 1.5 to 2.0 µg diclazuril equivalents/ml were observed at 6 hours after dosing. There was a rapid equilibrium between plasma and tissues, but the plasma concentrations were 2 to 10 times higher than the corresponding concentrations in tissues. Liver and kidney contained the highest quantities of residual radioactivity, while muscle and skin+fat contained much less.

    The elimination of radioactivity from plasma as well as from tissues was monophasic with a half-life of approximately 50 hours. All radioactivity in plasma was due to parent diclazuril for up to 72 hours after dosing. In liver parent diclazuril accounted for more than 90% of the residual radioactivity. About half of the dose was excreted within 24 hours, almost exclusively as unchanged diclazuril. At longer intervals after the dosing, gradually more transformation products were recovered in the excreta.

During the 10 experimental days more than 95% of the dose was excreted. A main degradation product accounted for 5.3% of the dose in the 0 to 96 hour excreta, whereas other diclazuril-related degradation products each accounted for less than 2%.

Twenty eight turkeys received a single oral dose of 1 mg/kg bw ^{14}C-diclazuril. Maximum radioactivity concentrations of 1780 ± 190 µg equivalents/litre were attained in plasma at 6 hours. Elimination proceeded with a half-life of approximately 38 hours. There was a rapid but limited distribution between plasma and tissues. The liver and the kidneys showed a maximum of 1400 ± 210 µg equivalents/kg and 1090 ± 140 µg equivalents/kg, respectively, at 6 hours. Skin+fat contained 568 ± 86 µg equivalents/kg and muscle 210 ± 46 µg equivalents/kg at maximum 6 hours after dosing. The depletion rate was similar in all tissues with a half-life in the range from 34 to 46 hours. Radiochromatograms of the extracts showed a large peak of the parent compound accounting for 98% of the radioactivity in liver samples after 6 hours and 85% for the livers sampled after 48 and 72 hours.

As for diclazuril, it can reasonably be assumed that in pigeons clazuril will not be extensively metabolised and that the parent drug is the marker residue.

12. A non-radiometric depletion study was carried out on 9 male and 9 female pigeons. As recommended for veterinary use, the pigeons received an oral tablet containing 2.5 mg clazuril (approximate dose of 5 mg/kg bw). At 1, 3, 5, 8, 24, 48, 96, 168 and 240 hours after administration, one male and one female pigeon were killed and the clazuril concentration was determined using a HPLC method with a quantification limit of 100 µg/l plasma or 100 µg/kg tissue. Overall, concentrations were higher in male than in female pigeons. A maximum plasma concentration of 14700 µg/l was obtained at 5 hours. At 8 and at 24 hours, a maximum concentration of 4600 µg/kg was measured in muscle while a maximum of 13200 µg/kg was seen at 24 hours in the liver. Over the 240 hours, the average clazuril concentration was 10% and 20% higher in male muscle and liver, respectively, than in the corresponding female tissues.

13. Assuming a daily consumption of pigeon of 300 g muscle and 100 g liver, the maximal total daily intake, 24 hours after treatment, is 2.7 mg corresponding to 90 % of the toxicological ADI.

14. A HPLC method has been developed to measure residues of clazuril in pigeon edible tissus and the limit of quantification was 100 µg/kg for liver and muscle.

Conclusions and recommendation

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

- clazuril is of low toxicity,
- the animals are unlikely to be sent for slaughter immediately after treatment,
- 24 hours after treatment the maximum daily intake of residues is below the ADI;

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

Pharmacologically active substance(s)	Animal species	Other provisions
Clazuril	Pigeon	