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

EMEA/MRL/314/97-FINAL
April 1998

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TOLTRAZURIL

SUMMARY REPORT (1)

1. Toltrazuril, a triazinetrione derivative, is intended for use in chickens and turkeys for the prevention and treatment of coccidiosis. Administration is via drinking water at 25 mg/l for continuous administration over 48 hours or at 75 mg/l given for 8 hours per day, on 2 consecutive days. Both treatments correspond to a dose of approximately 7 mg toltrazuril/kg bw/day. For curative treatment, one medication that can be repeated after 5 days is recommended and for preventive treatment of chickens, three medications on days 9 to 10, 16 to 17 and 23 to 24 of life.

2. Toltrazuril induces changes in the fine structure of coccidian development stages that are mainly due to a swelling of the endoplasmatic reticulum and of the Golgi apparatus and to abnormalities in the peri-nuclear space, disturbances in nuclear division. Toltrazuril leads to a reduction of enzymes of the respiratory chain of the parasites. The biochemical mode of action of toltrazuril which causes obstruction of the wallforming bodies of Eimerian macrogamonts can not be explained up to now.

3. After a single oral administration of 20 mg/kg bw of ^{14}C-toltrazuril to rats, the maximum plasma radioactivity, in the magnitude of 25 to 36 µg equivalents toltrazuril/ml, were reached 8 and 24 hours after administration in males and females, respectively. The elimination half-lives of radioactivity were significantly different according to the sex: 75 hours in females, 23 hours in males.

   83 to 90% of the radioactivity administered were excreted within 168 hours in the excreta, the faecal excretion accounting for 84% to 96% of the recovered radioactivity. Only 2 to 6% were excreted via urine.

   Unchanged toltrazuril was the main radioactive component detected in faeces, accounting for 64.4 to 92.8% of the faecal radioactivity. Four metabolites were identified in faeces. One major metabolite, toltrazuril-sulfone, represented 4.6 to 16.0% of the faecal radioactivity. Two other metabolites, the toltrazuril-sulfoxide and the sulfide of the hydroxymethyl analogue of toltrazuril accounted for less than 1%. The sulfone of the hydroxymethyl analogue of toltrazuril was only seen in male faeces.

   The main pathways of biotransformation proceeded via stepwise sulfoxidation and hydroxylation of the methyl group in the phenylene moiety.

   There is indication for an accumulation of toltrazuril and its main metabolites in the serum of female rats receiving repeated doses of 30 mg/kg bw toltrazuril.

4. Following repeated oral administrations to chickens of 8 mg of ^{14}C-toltrazuril/kg bw/day for 2 days, 50% of the total radioactivity administered was eliminated by 4.5 days after the first dose, increasing to 90% at 15.5 days. In serum, toltrazuril-sulfone accounted for 97 to 99% or all of the radioactivity at 4.5 or 8.5 days respectively after the end of the treatment. Of the nine metabolites isolated from chicken faeces, three major metabolites could be identified: toltrazuril, toltrazuril-sulfoxide and toltrazuril-sulfone accounting for 33.1%, 15% and 16.4% of the faecal radioactivity measured 24 hours after the end of the treatment.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Switchboard (44-20-7) 418 8400 Fax (44-20-7) 418 8447
E-mail: mail@emea.eudra.org http://www.eudra.org/emea.html

In plasma, the radioactivity levels ranged from 21 to 29 µg equivalents toltrazuril/ml after the end of the treatment. Then, the radioactivity declined to reach 1 µg/ml and 0.2 µg/ml at 8.5 and 14.5 days after the last administration, respectively.

5. Following repeated oral administrations to turkeys of 7 mg of non radiolabelled toltrazuril/kg bw/day for 2 days, toltrazuril concentrations in plasma were low (C_{max} equals 0.62 µg/ml after the last administration) and the parent compound could no longer be detected 24 hours after the end of the treatment. Toltrazuril-sulfoxide (C_{max} equals 2 µg/ml just after the end of the treatment) could not be detected 72 hours after the end of the medication. Toltrazuril-sulfone was the major residue found in plasma, persisting during 24 hours after the end of treatment at a steady state of 5 µg/ml and decreasing slowly to 2 µg/ml at 120 hours.

6. The acute oral toxicity of toltrazuril was low: LD_{50} close to 2000 mg/kg bw in rats and higher than 5000 mg/kg bw in mice.

7. In a three-month oral toxicity study conducted in rats, toltrazuril was administered at dose levels of 0, 15, 60 and 240 mg/kg feed (approximately 0, 1.1, 4.2 and 16.6 mg/kg bw/day in males and 0, 1.2, 4.7 and 17.4 mg/kg bw/day in females). At the highest dose, the weight gain and the daily food intake were significantly decreased. Slight effects on the haematological parameters and disturbances of the liver function were also observed. At 15 mg/kg feed variations in some parameters, although statistically significant were not considered relevant because they were not dose-related. The dose of 15 mg/kg feed (1 mg/kg bw/day) can be retained as the NOEL for this study.

   A second 3-month oral toxicity study was conducted in dogs treated with toltrazuril at doses of 0, 1.5, 4.5 and 13.5 mg/kg bw/day. The highest dosage induced a significant increase in the weight of the heart without any histological changes nor circulatory disturbance. The mean weight of the testes and the weight of the prostate were decreased. The NOEL retained was 1.5 mg/kg bw/day.

8. A 2-generation study in rats receiving toltrazuril in diet at doses approximately of 0, 0.3, 1.25 and 5 mg/kg bw/day showed that the number of still-born pups was increased in all treated groups, the statistical significance being only observed for the highest dosage and for the first generation of the lowest dose group. Due to these equivocal results, 0.3 mg/kg bw/day was retained as a LOEL.

9. Two teratogenicity studies were performed in rats. In the first study, Wistar strain rats received toltrazuril at doses of 0, 3, 10, 30 mg/kg bw/day (tylose suspension) and 0 and 1 mg/kg bw/day (in supplemental study). Teratogenicity and embryotoxicity such as dysplasia of long bones, hydrops, cleft palate, microphthalmia were observed at the highest dose. When compared to the controls, a statistical decrease in the number of live foetuses was reported at 3 and 10 mg/kg bw/day. As only a slight increase in resorption was seen at 1 mg/kg bw/day, a LOEL of 1 mg/kg bw/day was retained from this study.

   In a second study, Sprague Dawley rats were given 0, 1, 3, 10 and 30 mg/kg bw/day of toltrazuril (carboxymethylcellulose). In this study, NOELs of 3 mg/kg bw/day and 10 mg/kg bw/day were retained for maternotoxicity and embryotoxicity.

   Two other teratogenicity studies were conducted in rabbits. In the first study, the rabbits received toltrazuril at doses of 0, 1, 3 and 10 mg/kg bw/day. Significant increase in the number of abortions was reported at 3 and 10 mg/kg bw/day. As three litters showed runts at the lowest level, no NOEL was retained.

   In the second study, 0, 0.5, 0.75, 1 and 2 mg/kg bw/day of toltrazuril were given to rabbits. At 2 mg/kg bw/day, a significantly increased number of fused sternebrae was reported. As significant increase of placental weights as reported at 0.75 mg/kg bw/day and 1 mg/kg bw/day, the lowest dose 0.5 mg/kg bw/day was retained as the NOEL.

10. Toltrazuril gave negative results in five *in vitro tests* (Ames tests, mammalian cell mutation in Chinese hamster ovary (CHO) cells at the HGPRT locus, chromosomal aberrations test, Chinese hamster ovary (CHO) cells and in unscheduled DNA synthesis in rat primary hepatocytes) and in one *in vivo* test (*in vivo* micronucleus test after oral administration).

    A study on DNA-adduct formation in rat uterus after single oral doses of 300 or 600 mg/kg bw or repeated administrations of 30 mg/kg bw/day for 7 days gave negative results. As the test used is not validated and due to deficiencies of the study, these results should be taken with caution.

    It was concluded that toltrazuril was not mutagenic.

11. Carcinogenicity studies were performed in mice (approximately 0, 2, 6, 18 mg/kg bw/day) and in rats (approximately 0, 1, 3, 10 mg/kg bw/day).

    In mice, toltrazuril showed equivocal results as the significant increased incidence of lymphomas reported in males treated at the highest dosage was within the historical range of spontaneous rate (18% in males, 2% in control and 0 to 33% in the historical controls). The increase of incidence of lymphomas in females (34% in treated animals, 22% in control) was just above the upper limits of historical control (33%) and was not significant.

    In female rats, toltrazuril increased the incidence of endometrial adenocarcinomas significantly at the highest dosage (23/50 at 10 mg/kg bw/day; 6/50, 4/50 and 8/50 at 0, 1 and 3 mg/kg bw/day). At the highest dose, a reduction in the number of mammary tumours and hyperplasias in the pituitary was noticed in these rats. However, considering the significant increase in total incidence of pre-neoplastic and neoplastic lesions of the uterus for the two highest dosages (45 and 37 at 10 and 3 mg/kg bw/day versus 14 and 13 in the lowest and control groups), 3 mg/kg bw/day was retained as the threshold level for neoplastic tumours, while 1 mg/kg bw/day was retained as the threshold dose for tumour promotion, i.e. pre-neoplastic lesions.

    Imbalance in the female hormone system was suggested being involved in this tumourigen mechanism.

12. The assessment of the toxicity of toltrazuril-sulfone showed that this main metabolite is less toxic than the parent compound. This is reflected by the higher NOEL obtained in 3-month toxicity studies (11.2 mg/kg bw/day versus 1.1 mg/kg bw/day with toltrazuril in rats and 8.3 mg/kg bw/day versus 1.5 mg/kg bw/day with toltrazuril in dogs) and in teratogenic study in rats (30 mg/kg bw/day versus 1 mg/kg bw/day with toltrazuril). Two-generation-reproductive and carcinogenicity studies were not performed.

13. No antibacterial activity of toltrazuril was shown up to a concentration of 128 µg/ml on *E. Coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus,* S*treptococcus pyogenes.* The MIC values of toltrazuril against *Trichophyton mengrophytes* and *Microsporum canis* ranged at 4 and 64 mg/l. No activity was found against *Candida albicans, Aspergillus fumigatus* and *Penicillium communis*.

14. Considering that:

    - there is no evidence of genotoxic mechanism involved in the uterine tumour development,
    - rodents are known to be more sensitive to hormonal changes than humans,
    - the findings of the carcinogenicity study are regarded as a rat-specific,
    - the threshold dose of 1 mg/kg bw/day for pre-neoplastic lesions was retained from the carcinogenicity study in rats to establish the toxicological ADI,

    a toxicological ADI of 0.002 mg/kg bw/day, corresponding to 120 µg per person, was calculated by applying a Safety Factor of 500. The Committee considered this Safety factor appropriate to take into account the conservative approach of using the threshold dose with respect to pre-neoplastic lesions for the calculation of the ADI.

15. Among the seven depletion studies provided for chicken, two of them were conducted with the radioactively labelled compound.

In the radiometric studies, the animals received ^{14}C-toltrazuril on days 9 to 10, 16 to 17 and 23 to 24 at doses ranging from 7 to 10 mg/kg bw/day. At 0.25 days after the last dosing, the highest levels of radioactivity measured in liver and kidney were in the magnitude of 30000 µg equivalents toltrazuril/kg whereas in muscle they were close to 5000 µg equivalents toltrazuril/kg. At 8 days after the last dosing, significant levels of radioactivity were still quantified: 210, 430, 1480 and 1240 µg equivalents toltrazuril/kg in muscle, fat, liver and kidney respectively. Then, they declined to reach 50, 90 µg equivalents toltrazuril/kg in muscle and fat and 200 µg equivalents toltrazuril/kg in liver and kidney, 16 day post-dosing.

Toltrazuril and its two major metabolites, toltrazuril-sulfoxide and toltrazuril-sulfone, were measured.

Toltrazuril, the parent compound, represented 29 to 43% of the total radioactivity 0.25 days after the end of the treatment and insignificant percentage at the slaughtering time on the eighth day after the end of treatment.

Toltrazuril-sulfoxide could only be quantified at 0.25 day post treatment in all tissues and accounted for less than 4% in liver at 8 days after the end of the treatment.

Toltrazuril-sulfone was the major metabolite. Eight days after the end of the treatment, it represented 100% of the total radioactivity in muscle and fat and 88% in liver.

Among all the non-radiometric studies, the results of one of them was considered as relevant to establish the MRLs. Chickens received toltrazuril at doses of 14.1 mg/kg bw/day on 2 consecutive days per week, three times at one week interval.

One day after the end of the treatment, the concentrations of toltrazuril were 342, 1845, 870, 1332 and 1077 µg toltrazuril/kg in muscle, fat, skin, liver and kidney. Then, they declined to reach, 6 days after dosing, less than 10, 81, 33, 22, 15 µg toltrazuril/kg in muscle, fat, skin, liver and kidney respectively. Ten days, after dosing, toltrazuril could only be detected in fat and skin (24 and 11 µg/kg)

One day after the end of the treatment the residues of toltrazuril-sulfoxide were 773, 1268, 1030, 3416, 4411 µg/kg in muscle, fat, skin, liver and kidney. Then they declined to reach, 6 days after dosing, approximately the limit of quantification (10 µg toltrazuril-sulfoxide/kg) in muscle, fat and skin whereas concentrations of 54 and 95 µg toltrazuril-sulfoxide/kg were still measured in liver and kidney. Ten days after dosing, toltrazuril-sulfoxide could only be detected in liver and kidney (16 and 36 µg/kg).

The concentrations of toltrazuril-sulfone were much higher than those measured for the other metabolites: 4742, 13267, 7931, 21275, 17084 µg/kg in muscle, fat, skin, liver and kidney at one day post dose. Sixteen days after dosing large amounts were still measured in edible tissues: 38, 104, 97, 225, 152 µg toltrazuril-sulfone/kg in muscle, fat, skin, liver and kidney. Twenty days after dosing, significant amounts of toltrazuril-sulfone were still measured in liver and kidney, 117 and 152 µg/kg, respectively, whereas in other tissues they ranged between 30 to 85 µg/kg.

16. In the two depletion studies furnished in turkeys, the profile of toltrazuril depletion in edible tissues was the same than that observed in chickens. In a main study, the turkey received, via drinking water, 8.2 mg of toltrazuril/kg bw per day for two days per week, three times at one week interval.

    Two days after the end of the treatment, the concentrations of toltrazuril were below the limit of quantification in muscle and close to 100, 75, 50 and 40 µg/kg in fat, skin, liver and kidney. Six days post dose, toltrazuril could only be measured in fat (23 µg/kg).

Two days after the end of the treatment, the levels of toltrazuril-sulfoxide were 59 µg/kg, 374 µg/kg and 491 µg/kg in muscle, liver and kidney, respectively, and close to 120 µg/kg in skin and fat. Then, they declined to be in the magnitude of the limit of quantification for all edible tissues except fat (45 µg/kg), 8 days post dose.

The concentrations of toltrazuril-sulfone were close to 2140, 8140, 3890, 10750 and 9500 µg/kg in muscle, fat, skin, liver and kidney at two-day post dose. Fourteen days after dosing, 44, 113, 81, 259, 178 µg/kg were measured in muscle, fat, skin, liver and kidney, respectively. Twenty-one days after dosing, significant amounts of toltrazuril-sulfone were still measured in all edible tissues and were in the magnitude of 50 µg/kg for muscle, 100 µg/kg for skin, of 200 to 250 µg/kg for fat, kidney and liver.

17. A fully validated analytical method for monitoring residues of toltrazuril, toltrazuril-sulfoxide and toltrazuril sulfone in edible tissue of chicken and turkey was provided. The limit of quantification is 10 µg/kg for toltrazuril and toltrazuril-sulfoxide and 20 µg/kg for toltrazuril-sulfone.

Conclusions and recommendation

- Having set a toxicological ADI of 0.002 mg/kg bw, i.e. 120 µg for a person of 60 kg bw,
- having considered the distribution profile of residues of toltrazuril-sulfone in edible tissues at 16-day or 14-day slaughtering time, for chicken and turkey respectively,
- having considered that toltrazuril-sulfone is the most appropriate marker residues as it represents about 100% of the total residues at these slaughtering times for both species,
- having considered that the physico-chemical analytical methods available to measure residues of in edible tissues of turkey and chicken are fully validated;

the Committee recommends the inclusion of toltrazuril in Annex I of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Toltrazuril	Toltrazuril sulfone	Chicken	100 µg/kg 200 µg/kg 600 µg/kg 400 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption
		Turkeys	100 µg/kg 200 µg/kg 600 µg/kg 400 µg/kg	Muscle Skin + fat Liver Kidney	

Based on these MRLs values, the daily intake will represent about 93% of the toxicological ADI.