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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TOLTRAZURIL (Extension to pigs)

SUMMARY REPORT (2)

1. Toltrazuril is a triazinetrione derivative used as anticoccidial agent. It is widely used in chicken and turkeys for the prevention and treatment of coccidiosis, by administration via drinking water.

   A toxicological ADI of 0.002 mg/kg bw (i.e. 0.12 mg/person) has previously been established by the Committee for Veterinary Medicinal Products.

   Currently, toltrazuril is included 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
		Turkey	100 µg/kg 200 µg/kg 600 µg/kg 400 µg/kg	Muscle Skin + fat Liver Kidney	

   An application has now been submitted for an extension of the MRLs to pigs. Toltrazuril is intended to be administered as an oral suspension containing 5% toltrazuril at a single oral dose of 20 mg/kg bw in the first week of life of piglets.

2. In a radiolabelled pharmacokinetic study in pigs, after a single oral administration of 20 mg/kg bw of ^{14}C-toltrazuril, the radioactivity in plasma increased from 3002 µg equivalents/kg at 3 hours after dosing, to a maximum of 13 000 µg equivalents/kg at 2 days post dosing. The radioactivity levels declined to 11 130, 2122 and 164 µg equivalents/kg at 6, 28 and 72 days post administration, respectively. The half-life of elimination was 148.2 hours.

   The plasma toltrazuril concentrations rose from 2919 µg/kg at 3 hours after dosing to a highest mean concentration of 7688 µg/kg, 2 days post dose. Then the concentrations of toltrazuril declined to 3289 µg/kg and 871 µg/kg at 6 and 14 days post dosing, respectively. At later sampling times toltrazuril could not be detected in plasma.

   The toltrazuril sulfoxide concentration in plasma was 981 µg/kg at 0.5 day post dosing, then reached a mean plateau in the magnitude of 3200 µg/kg at 2 to 3 days post dose, and declined to 2185 µg/kg and to 443 µg/kg at 6 and 14 days post dosing, respectively. At later sampling times toltrazuril sulfoxide could not be detected in plasma.

   In plasma, toltrazuril sulfone was measured 1 day post administration (443 µg/kg) and its concentration increased to 6356 µg/kg at 14 days post dosing, then declined to 2055 µg/kg and 154 µg/kg at 28 and 49 days post dose, respectively.

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

Toltrazuril was the major plasma component up to 3 days (99% of the plasma radioactivity, immediately after the oral dose), declining to 48.6% and 10.75% at 3 and 14 days post dosing, whilst toltrazuril sulfone increased from 47.58% of the plasma radioactivity at 6 days post dosing to 100% at 49 days after the oral administration.

3. In a non-radiolabelled pharmacokinetic study, 2 groups of 6 piglets (14 day-old) received a single administration of 20 mg/kg bw toltrazuril either by oral or intravenous route.

   After intravenous administration, the mean concentration of toltrazuril in the serum declined from 16 210 µg/kg at 1 hour after the administration, to 14 710 µg/kg, 2651 µg/kg, 211 µg/kg and 88 µg/kg at 1, 7, 21 and 28 days post dose, respectively.

   Toltrazuril sulfoxide could be measured in serum 1 hour post injection (368 µg/kg), reached a mean peak of 4734 µg/kg at 2 days post injection and declined to 1510 µg/kg, 67 µg/kg and 38 µg/kg at 7, 21 and 28 days post dose, respectively.

   Toltrazuril sulfone could be measured in serum 1 hour post injection (66 µg/kg), reached a mean peak of 12 045 µg/kg at 9 days post injection and declined to 11 461 µg/kg, 7084 µg/kg and 3496 µg/kg at 14, 21 and 28 days post dose, respectively.

   After oral administration, the serum concentration of toltrazuril was 1900 µg/kg at 1 hour. The highest mean concentration measured was 7539 µg/kg at 2 days post dose.Then the concentration declined to 2079 µg/kg, 96 µg/kg and 20 µg/kg at 7, 21 and 28 days post dosing, respectively.

   Toltrazuril sulfoxide could be measured in serum 1 hour post injection (20 µg/kg), reached a mean peak of 3208 µg/kg at 2 days post dosing and declined to 1228 µg/kg, 31 µg/kg and 15 µg/kg at 7, 21 and 28 days post dose, respectively.

   Toltrazuril sulfone could be measured in serum 1 hour post injection (25 µg/kg). It remained at high concentrations from 7 to 14 days post administration (6200 to 6959 µg/kg). Then the concentration of toltrazuril sulfone declined to 4982 µg/kg and 3276 µg/kg at 21 and 28 days post dose, respectively.

   The oral bioavalability for toltrazuril, toltrazuril sulfone and toltrazuril sulfoxide was 69%, 62.5% and 66.4% respectively. After oral administration, the mean serum half-lives were 3 days, 16.9 days and 2.8 days for toltrazuril, toltrazuril sulfone and toltrazuril sulfoxide, respectively.

4. Following oral administration of 20 mg ^{14}C-toltrazuril to piglets, the elimination of the radioactivity in excreta was slow: 6.88%, 13% and 25% were recovered at 6, 8 and 11 days post dosing. By 21 days post dosing 49% of the administered radioactivity were recovered, the major route of excretion being via faeces (73.33% of the recovery radioactivity). Urine contained 24.08% of the excreted radioactivity.

   Samples of faeces contained 2 major compounds, the parent compound and its sulfone and two minor unknown compounds. The percentage of toltrazuril sulfone in the faecal radioactivity increased from 2.23% at 4 days post dosing to 71.27% after 21 days, while that of toltrazuril decreased from 83.75% at 4 days after the administration to 12.876% after 21 days.

   Seven components were found in urine: toltrazuril, toltrazuril sulfone, toltrazuril sulfoxide, 3 compounds for which the chemical name was not given, i.e. WAK 5693, WAK 5665-2 and SJJ 5441 (hydroxymethyl derivative), and a further unknown.

   Only a small fraction of parent compound was measured in urine. The percentages of the different metabolites varied according to the time. At 14 days post dosing toltrazuril, toltrazuril sulfone, toltrazuril sulfoxide, WAK 5993, WAK 5665-2 and SIJ 5441 represented 5.49%, 18.84%, 7.69%, 3.09%, 29.33% and 12.38% of the urinary radioactivity, respectively. Toltrazuril, toltrazuril sulfone and WAK 5665-2 accounted for 0%, 26.93% and 49.24% of the urinary radioactivity 21 days after the administration.

5. Toltrazuril is metabolised by stepwise sulfoxidation, producing sulfoxide and sulfone derivatives, and to a minor extent by hydroxylation. Toltrazuril metabolism can be considered as qualitatively similar in chicken/turkeys, rats and pigs.

6. Toltrazuril orally administered as a 5% suspension at doses up to 100 mg/kg bw is well tolerated in 7 day old piglets.

7. In a radiometric depletion study, 4 groups of 4 piglets (5-day old, JSR/Large White) received a single oral administration of 20 mg/kg bw of ^{14}C-toltrazuril. Animals were sacrificed at 14, 28, 49 and 70 days after the administration. The total radioactivity was quantified in all edible tisues. Toltrazuril, toltrazuril sulfone and toltrazuril sulfoxide were also separated and quantified.

   Fourteen days after a single oral administration of 20 mg/kg bw of ^{14}C-toltrazuril, the mean total radioactivity levels were 3134 µg equivalents/kg in muscle, 5963 µg equivalents/kg in fat, 4373 µg equivalents/kg in skin, 9544 µg equivalents/kg in liver and 5814 µg equivalents/kg in kidney. Then, they declined to 1076, 2061, 1827, 4255 and 2532 µg equivalents/kg in muscle, fat, skin, liver and kidney, respectively, by 28 days post-dosing. Edible tissues still contained significant amounts of radioactivity 49 days post dosing with 54, 96, 128, 332 and 207 µg equivalents/kg in muscle, fat, skin, liver and kidney, respectively, and 14, 26, 77, 100 and 71 µg equivalents/kg in muscle, fat, skin, liver and kidney at 70 days post administration.

   The concentrations of the parent compound declined from 65, 967, 752, 971 and 521 µg/kg in muscle, fat, skin, liver and kidney, 14 days post dose to 10, 77, 70, 82 and 33 µg/kg in muscle, fat, skin, liver and kidney, 28 days post dose. It could not be detected at later sampling times.

   Toltrazuril sulfoxide could only be quantified at 14 days post administration in muscle (632 µg/kg), liver (329 µg/kg) and in kidney (228 µg/kg). It could not be detected at later sampling time points.

   Toltrazuril sulfone could be determined up to 70 days post dosing. Fourteen days after administration, the following concentrations were measured: 2294 µg/kg in muscle, 4878 µg/kg in fat, 3464 µg/kg in skin, 7794 µg/kg in liver and 4105 µg/kg in kidney. They declined to 1061 µg/kg in muscle, 1692 µg/kg in fat, 1523 µg/kg in skin, 4055 µg/kg in liver and 2019 µg/kg in kidney by 28 days post dosing. Forty-nine days post dosing, 54, 98, 116, 308 and 139 µg/kg of tolrazuril sulfone were quantified in muscle, fat, skin, liver and kidney, respectively. At 70 days post administration, toltrazuril sulfone could not be detected in muscle and fat, whereas liver and kidney concentrations of toltrazuril sulfone were 94 µg/kg and 35 µg/kg, respectively.

8. Toltrazuril sulfone was the major metabolite measured in edible tissues of pigs and accounted for nearly all the radioactivity 28 days after treatment. At 28 days post dosing, toltrazuril sulfone accounted for 98.6% of the total radioactivity in muscle, 82.5% in skin + fat, 95.3% in liver and 79.7% in kidney. At 49 days toltrazuril sulfone accounted for 100% of the total radioactivity in muscle and in skin + fat, for 92.73% in liver and for 67.1% in kidney. At later sampling time points, the concentrations in the edible tissues were too low to estimate correctly the percentages.

9. In a non-radiometric depletion study, groups of 4 piglets (3-day old, meat hybrid, Pietrain-Hampshire cross) received a single oral administration of 20 mg/kg bw of toltrazuril. Animals were sacrificed at 14, 28, 49, 70, 91 and 112 days after the administration.

   Toltrazuril and toltrazuril sulfoxide were found in small quantities at 14, 28 and 49 days post dosing. At later sampling time points, the concentrations were below the limit of quantification (less than 10 µg/kg).

   At 14 days after dosing, the mean concentrations of toltrazuril sulfone were 3282 µg/kg in muscle, 7187 µg/kg in fat, 5121 µg/kg in skin, 9022 µg/kg in liver and 5239 µg/kg in kidney. They then declined to 1268, 2941, 2283, 5698, 2728 µg/kg in muscle, fat, skin, liver and kidney, 28 days post dosing, respectively. At 49 days after dosing, the concentrations of toltrazuril sulfone were 223 µg/kg in muscle, 331 µg/kg in fat, 279 µg/kg in skin, 1019 g/kg in liver and 516 µg/kg in kidney. At 70 days post dosing, toltrazuril sulfone could only be quantified in fat (28 µg/kg), in liver (50 µg/kg) and in kidney (27 µg/kg). At later sampling time points, no toltrazuril sulfone could be measured in the edible tissues (less than 20 µg/kg).

10. The HPLC analytical method based on fluorescence detection proposed to monitor residues of toltrazuril in edible tissues of pigs was not fully validated according to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community. The limits of quantification for toltrazuril sulfone, established according to the requirements of Volume VI are 20 µg/kg for all edible tissues, the limits of detection being 13 µg/kg for muscle and liver, 15 µg/kg for kidney, 10 µg/kg for fat and 11 µg/kg for skin. The values for skin + fat in natural proportion were not provided.

Conclusions and recommendation

Having considered that:

- the toxicological ADI is 0.002 mg/kg bw (i.e. 120 µg/person),
- the tissue distribution of residues at 49 days is most appropriate as the basis of the MRLs,
- the toltrazuril sulfone was retained as the suitable marker residue,
- 49 days after the end of the treatment, the percentage values of toltrazuril sulfone towards total residues is known for all edible tissues and equals 100% for muscle and skin + fat, 92.7% for liver and 67% for kidney,
- an analytical method, based on HPLC with fluorescence detection, for the routine determination of toltrazuril sulfone in edible tissues of pigs was provided,

the Committee for Veterinary Medicinal Products recommends the inclusion of toltrazuril for pigs in Annex III 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	Porcine	100 µg/kg 150 µg/kg 500 µg/kg 250 µg/kg	Muscle Skin + fat Liver Kidney	Provisional MRLs expire on 1.1.2001

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

Before the Committee can consider the inclusion of toltrazuril for pigs in Annex I of Council Regulation (EEC) No 2377/90, the points included in the list of questions should be addressed.

LIST OF QUESTIONS

1. The applicant should complete the validation of the analytical method for monitoring purposes, i.e. for measuring the marker residue in porcine edible tissues including the combined target tissue skin + fat in natural proportions, according to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community. The method for all edible porcine tissues should presented in a single volume according to an internationally recognized format (e.g. ISO 78/2). Chapter 2 of the ISO format 78/2 «warning and safety precautions» should also be completed to underline the danger to be encountered by the analyst during the procedure due to the presence of dichloromethane.