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

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ENILCONAZOLE

SUMMARY REPORT

1. Enilconazole (synonym: imazalil), (±)-1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-lH-imidazole (CAS No 73790-28-0) is a broad-spectrum antimycotic, belonging to the imidazole group group that, in contrast to the nitro-imidazole molecules, lacks the nitro group. In veterinary medicine, enilconazole is used as a topical antimycotic (4 mg/kg bw) against dermatophytes in cattle, horses and dogs. In several countries, it is also used as a fungicide formulation for the disinfecting of farm buildings including poultry houses.

2. Enilconazole is an external antimycotic substance with a broad spectrum activity against dermatophytes (*Microsporum sp.*, *Trichophyton sp.*). It has some activity against *Aspergillus sp.*, *Penicillium sp.* and yeasts such as *Candida sp.*. There is also a certain bactericidal activity but not against *Escherichia coli* end *Pseudomoniae*. Its mode of action involves inhibition of the fungal cytochrome P_{450} dependent 14α-dimethylation of lanosterol to ergosterol, a specific component of the membranes for fungi and yeasts.

3. The pharmacokinetics of enilconazole were studied in laboratory animals (rat, mouse, rabbit and dog). Radiolabelled studies in rats indicated rapid absorption by oral administration. After gavageadministration of ^{14}C-enilconazole at 2.5, 10 or 40 mg/kg to rats, enilconazole represented on average only 0.25% of the plasma radioactivity at 2.5 mg/kg, 3.0% at 10 mg/kg and 16.7% at 40 mg/kg. In rats, the radioactivity absorbed in plasma decreased with an apparent half-life of 4 to 5 hours after oral doses of 2.5, 10, and 40 mg/kg. The half-life of the parent substance was about 1 hour, independently of the dose. Approximately 5% of total radioactivity was retained in faeces. Sixty-three percent of the radioactivity was excreted during the first day, another 18% of total radioactivity during the second day. Total residual amounts in rats were estimated at 6 and 3% of the administered dose, two and four days after treatment respectively. Metabolism in the rat was extensive, only 10% of the urinary excretion was due to the unchanged compound. Accumulation in fatty tissue did not occur. In a similar study where rats were traeted orally with ^{3}H-enilconazole, no unchanged compound was found in the urine, in the faeces the unchanged compound accounted for only 0.1% of the dose.

4. The absolute bioavailability of enilconazole was studied in three cows by measuring the plasma levels following oral, topical and intravenous doses of 4 mg/kg. After intravenous dosing, the plasma levels decayed triexponentially with a terminal half-life of 11 hours. Peak levels after oral and topical dosing were attained within 1 hour (17.1 ng/ml and 48.5 ng/ml) and levels decreased rapidly. After oral administration, the average area under the curve (AUC) value was 62 ng.h/ml and represented 1.4% of that of an equal intravenous dose (due to an extensive first-pass metabolism). The average AUC value was 230 ng.h/ml after topical administration and represented 5% of that after intravenous dosing (due to a limited absorption). One of three cows had an absolute bioavailability after topical administration of 9.2% of the bioavailability after intravenous dosing.

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5. Two groups of cattle (one with normal (n=3) and one with scarified skin (n=3)) and one group of horses (n=3) were treated with a 4 mg/kg dose of enilconazole sprayed on the skin. Plasma levels of enilconazole were determined up to 48 hours after application (limit of detection 0.5 ng/ml). In all groups peak levels were attained within 1 hour and varied from 23 to 104 ng/ml in cattle and from 7 to 18 ng/ml in horses. Plasma levels were not detectable after 8 and 24 hours in horses and cattle, respectively. The absorption through scarified skin was similar to that through normal skin. The AUC values were on average 7 times higher in cattle than in horses (224 ng.h/ml for cattle with normal skin, 217 ng.h/ml for cattle with scarified skin versus 31 ng.h/ml for horses).

6. The metabolism of enilconazole is extensive. A large number of metabolites (at least 35) could be detected after oral administration, each representing less than 5% of the total radioactive residue. *In vitro* tests using microsomal fractions of various animal livers showed large species differences in the rate of metabolism of the drug. The metabolism rate was lowest in cattle increasing in the following order for the other species examined: dogs, rabbits, chicken, goats, and was highest in rats. This was confirmed *in vivo*, where elimination half-lives of the metabolites in cow liver were about twice those in goat liver after oral administration.

7. Enilconazole, even when administered by inhalation or by the intraperitoneal route shows a rather low acute toxicity with a large safety margin. The LD_{50} by oral administration was 390.7 mg/kg in male mice and 620.5 mg/kg in female mice. Both in female as in male rats, the LD_{50}s found in several studies were approximately 300 mg/kg. In one rat study this oral LD_{50} was found to be 1310 mg/kg in male and 403 mg/kg in female Wistar rats. The acute dermal LD_{50} was found to be 4200 mg/kg for male rats and 4880 mg/kg for female rats.

8. Repeated dose toxicity was carried out by oral administration in mouse, dog and rat (dosage range: 1.25, 2.5, 5, 10, 20, 40 and 80 mg/kg bw) during 3 to 24 months of which the dog study during 24 months showed as lowest level of toxicity the 5 mg/kg dose. A slight decreased appetite and a decreased body weight gain was observed in rats and dogs. The different studies identified the liver as the main target organ for toxicity, the observed liver changes included centri-lobular swelling, fatty surcharge and more numerous vacuoles at 20 and 80 mg/kg bw. In the dog a NOEL of 2.5 mg/kg bw was identified whereas in rats the NOEL was found to be 5 mg/kg bw.

9. Several multigeneration studies carried out in the rat with dose levels at 5, 20 and 80 mg/kg bw, showed only as a maternal effect an increase of gestation duration and as a foetal effect a decrease of the number of live pups and an increase of the number of still born pups, both at doses of 80 mg/kg. No teratogenicity could be demonstrated at any dose. In one rabbit study using orally administered dosages (0.63 and 2.5 mg/kg bw), a decreased bodyweight in dams and a decrease in litter size and survival in pups was observed, presumably as a result of the maternal toxicity. However, in three additional studies in rabbits using oral dosages ranging from 1.25, 2.5, 5, 10 to 20 mg/kg, a decreased body weight gain and food consumption in dams were observed at 10 and 20 mg/kg. At the highest dosage a decreased litter size and an increase in embryonal resorption was also recorded. The results do not allow to establish a NOEL for maternal toxicity in rabbits. However, the overall data clearly indicate that there is no direct embryotoxic or foetotoxic effect of enilconazole.

10. Five *in vitro* (DNA repair test in *Escherichia coli* (SOS), unscheduled DNA synthesis (UDS) test on rat hepatocytes, Ames reverse mutation test with *Salmonella typhimurium*, a chromosomal aberration test with human lymphocytes and a point mutation assay (HPRT locus) in V79 Chinese hamster lung cells) and 3 *in vivo* assays (sex-linked recessive lethal test on *Drosophila melanogaster*, micronucleus test in rats and mice and the dominant lethal test in mice) for primary DNA damage, point- and gene mutations, and chromosome aberrations did not show any mutagenic activity of enilconazole.

Imidazoles, to which parconazole belongs, have a different mode of action than nitro-imidazoles. Due to reduction of the nitro group, nitro-imidazole molecules induce short-live intermediates or free radicals that produce DNA damage by interacting with DNA and possibly other molecules. This mechanism is thought to have a role in the mutagenic and carcinogenic effect, which are characteristic for the nitro-imidazoles. This mechanism however, does not occur for the imidazole molecules, for which the mode of action involves inhibition of the fungal cytochrome P_{450} dependant 14α-dimethylation of lanosterol to ergosterol, a specific component of the membranes for fungi and yeasts.

11. During carcinogenicity studies in mice by oral route (2.5 to 130 mg/kg bw/day during 18 to 24 months) and in rats (1 to 40 mg/kg bw/day during 24 to 30 months) there was a significant increased incidence of liver tumours (hepatocellular adenomas) in male mice at 33 to 100 mg enilconazole/kg bw/day and in female mice at 130 mg enilconazole/kg bw, but not at 8 mg/kg bw in male mice and 40 mg/kg bw in female mice. Taken into account that the liver adenomas only occurred in mice at high dose levels and did not appear in rats and that enilconazole has shown no signs of mutagenicity, it was considered that enilconazole is unlikely to be of carcinogenic risk for humans.

12. Local tolerance studies in rabbits showed that enilconazole has only a very moderate to minimal irritating and a weak sensitising potential. Also tests carried out with different formulations in man showed no irritation or sensitisation.

13. Based on the NOEL of 2.5 mg/kg for the dog repeated dose toxicity study and using a safety factor of 100 a toxicological ADI of 0.025 mg/kg bw/day corresponding to 1.5 mg/kg person/day can be proposed.

14. Radiolabelled enilconazole was administered orally (0.665 mg/kg) twice a day to one lactating goat, in total 7 administrations. No unchanged drug could be detected, in plasma, or in milk. The largest part of the metabolites was hydrophilic compounds. No single compound could be identified as a marker residue. The parent drug was detectable in all edible tissues, except for milk but only at low percentages of the total residue (3.6% to 6.6%).

15. Metabolic characterisation was performed in edible tissues 16 hours after the last oral administration to a goat of 5.2 mg/kg radiolabelled enilconazole (twice daily for three days). Except in the milk, parent compound occurred in significant amounts in the organic extracts of liver, kidney, muscle and fat. In milk, except for one unknown major metabolite fraction (11%), eighteen metabolite fractions occurred in minor amounts below 8%. In liver, besides the parent compound (6%), fifteen minor metabolite fractions were detected below 5%. In kidney, besides the parent compound (4%), another metabolite occurred as major metabolite fraction, seven additional minor metabolite fractions below 5% were found. In muscle, besides the parent compound (3%), 2 other metabolites were detected as major ones. In fat, one major metabolite (25%) was identified.

16. One goat received twice daily for three days an oral ^{14}C enilconazole treatment at a dosage of 5 mg/kg bw. Attempts were made to additionally characterise and identify the ^{14}C enilconazole related radioactivity in the liver, kidney and milk of a goat. Unchanged enilconazole was only detected in the enzyme-hydrolysed aqueous phases of milk, liver and kidney and accounted for 0.3 to 0.7% of the original sample radioactivity. A metabolite that accounted for 11% of the original sample radioactivity was detected in the aqueous phase of milk.

17. The concentration profile of radioactivity in milk and the excretion pattern via urine and faeces was followed during 6 oral administrations of 5.2 mg/kg of ^{14}C-labelled test article to 1 goat. In milk radioactivity levels gradually increased from 0.406 mg/kg after the first to 0.826 mg/kg after the third administration. After the fourth to sixth administration, steady state levels were reached at 0.968 to 0.991 mg/kg. 0.1% of the radioactivity was excreted in the milk. At sacrifice (16 hours after the last administration), highest radioactivity levels were found in the excretory organs liver (19.8 mg/kg) and kidney (9.603 mg/kg). Lowest radioactivity levels were found in muscle (362 µg/kg) and fat (91 µg/kg).

18. Two milking cows were treated orally with enilconazole in three periods of five days each separated by a 72 hours drug-free period, first with 9 dosages of 0.20 mg, followed by 9 dosages of 0.67 mg and in the third period 9 dosages of 2 mg were administered. Plasma samples were taken before, during and after each ingestion period. Morning and evening milk samples were collected during the entire experiment. Plasma levels never exceeded 0.05 µg/ml. Plasma levels of two other metabolites was always lower then 0.01 and 0.02 µg/ml respectively. Enilconazole was only detected in the milk of the second cow at a concentration up to 7 µg/l. The total excretion of enilconazole in the milk was estimated as less than 0.4 mg (0.003% of the total dose) for the first cow and less than 0.64 mg (0.006% of the total dose) for the second cow. Milk concentrations of the two metabolites were always lower than the limit of detection of 5 µg/l.

19. Three groups of each three cows received nine oral dosages of enilconazole, twice daily at 0.2 mg/kg (group 1), 1.2 mg/kg (group 2) and at 4 mg/kg (group 3). Enilconazole milk levels could be detected above the limit of detection of 2 µg/l only in group 2 (14 µg/l) and in group 3 (32 µg/l). Steady state levels were reached after 2 to 4 doses. The metabolites R14821 (a-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol) and R42243 (1-[2-(2,4-dichlorophenyl)-2-(2,3-dihydroxy-propoxy)ethyl]-1H-imidazole) were found only in group 3 respectively around the limit of detection (2 µg/l) and at ± 20 µg/l. Total excretion of enilconazole with the milk was maximally 0.01% of the dose. At 24 hours after the last treatment a dose-linearity was observed for the enilconazole levels in the liver at 0.12, 0.42 and 1.05 mg/kg in group 1, 2 and 3. Metabolite levels were more than twice smaller than the levels of the parent drug. Only in the highest dosage group, enilconazole was detected in muscle, fat and kidney at concentrations of respectively 5, 44 and 20 µg/kg. In the 4 mg/kg group, liver enilconazole levels decreased with a half-life of 12.4 hours, those of two other metabolites with half-lives of 14 and 15.8 hours respectively.

20. The absorption and milk excretion of enilconazole and two of its basic metabolites were studied in six lactating cows, topically treated with 2-gram doses enilconazole. At 2 hours after dosing, peak plasma levels amounted to 22 ±10 µg/l. The elimination of the drug from the plasma occurred with an apparent half-life of 6 hours, suggesting a rather slow absorption of the drug. At 11 hours after treatment, the average milk concentration was 16 ±8 µg/l. Residual levels of enilconazole in liver, kidney, muscle and fat were measured 24 hours after topical application of a 2 gram dose of enilconazole to three calves. The enilconazole levels were found to be lower than 10 µg/kg. In these studies, the two basic metabolites of enilconazole, R14821 and R42243, could not be detected in any plasma, milk or tissue samples (concentrations lower than 5 µg/l for plasma and milk and 5 µg/kg for tissues).

21. One lactating cow was treated topically with 2 grams of enilconazole after the morning milking. Unchanged enilconazole was found in the milk at concentrations of 9 µg/l at 2 hours post treatment, 20 µg/l (4 hours), 12 µg/l (6 hours) and 7 µg/l at 8 hours. The milk concentrations after 8 hours until 48 hours post treatment were below the limit of detection of 2 µg/l. Two metabolites, R14821 and R42243 could not be detected in the milk during the course of the experiment at a limit of detection of 10 µg/l.

22. Eight lactating cows received four topical treatments at 72 hour-intervals with 2 g enilconazole (4 to 4.5 mg/kg). Milk samples were obtained before and during treatment and up to 79 hours after the last application. Enilconazole was detected in milk samples only at 7 hours after the last application (1.1 to 3.6 µg/l, limit of detection was 1 µg/l).

23. The analytical method provided for the determination of enilconazole and two metabolites (R14821 and R42243) in bovine edible tissues and milk was based on gas chromatography with electron capture detection with a quantification limit of 2 µg/l of milk and 2 µg/kg of tissue for enilconazole and R14821, and 10 µg/l of milk and 10 µg/kg of tissue for R42243.

24. Enilconazole is also used as a pesticide and MRL's for imazilil (enilconazole) in animal products were fixed at the lower limit of analytical determination by Council Directive 93/57/EEC, as no residues were expected to occur in products of animal origin as a result of consumption of feedstuff treated with imazalil (enilconazole). This is equivalent to a no residue situation, which appears to be compatible with the inclusion of enilconazole in Annex II of Council Regulation (EEC) No 2377/90.

25. In the assumption that edible tissues will contain 10 µg/kg and milk will contain 2 µg/l of the parent drug enilconazole at 24 hours after topical administration to cows and in the assumption that the parent drug only represents 5% of the total enilconazole related residue content, the maximum daily intake becomes 160 µg which is only 11% of the toxicological ADI.

Conclusions and recommendation

Having considered that:

- there is only limited absorption after topical administration,
- enilconazole is extensively and rapidly metabolised and rapidly eliminated,
- at 24 hours after topical administration, the amount of residues susceptible to be ingested by consumers represents only a low fraction (less than 11%) of the ADI,
- animals are unlikely to be sent for slaughter immediately after treatment;

the Committee concludes that there is no need to establish an MRL for enilconazole 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
Enilconazole	Bovine, equidae	For topical use only