

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

EPRINOMECTIN

SUMMARY REPORT (1)

1. Eprinomectin is a semi-synthetic compound of the avermectin family, intended for the treatment of internal and external parasites in cattle and in lactating cows. Eprinomectin is a mixture of two homologues, eprinomectin B1a (90%) and eprinomectin B1b (10%), which differ by a methylene group in the C25.

The recommended dosage regimen is a single dose of 0.5 mg/kg bw (0.1 ml/10 kg bw) applied topically along the midline of the animal's back.

2. The precise mode of action of eprinomectin remains unknown, despite extensive investigations with a variety of compounds from the same class.
3. In rats, after an oral administration of eprinomectin containing $^3\text{H}/^{14}\text{C}$ -dual labelled eprinomectin B1a at a level dose of 15.5 mg/kg bw, only 0.5% of the labelled part of the dose was recovered in urine and 76-99% in faeces. In faeces, the parental homologue eprinomectin B1a accounted for approximately 82% of total radioactivity. Five minor metabolites of eprinomectin B1a (1-6.5 %) were detected in faeces : M1, the 24a-hydroxymethyl metabolite, M2, the 24a-hydroxy metabolite, M3, the 26a-hydroxymethyl metabolite and M5, the N-deacetylated metabolite.

After oral repeated administrations of ^3H -eprinomectin, eprinomectin B1a and eprinomectin B1b being radiolabelled, at a level dose of 6 mg/kg bw/day for 7 consecutive days, it was shown that eprinomectin was metabolised more extensively in female rats than in male rats : the sum of eprinomectin B1a and eprinomectin B1b accounted 59.2 % in males and 20.9 % in females whereas M5 accounted 20.1% in males and 62.8% in females, 4 days after the end of the administration.

4. In rats, 48 hours after a single oral administration of 15.5 mg/kg bw eprinomectin containing $^3\text{H}/^{14}\text{C}$ -dual labelled eprinomectin B1a, significant amounts of ^{14}C residues were measured in tissues : 240 μg equivalent eprinomectin/kg in muscle, 570 μg equivalent eprinomectin/kg in fat, 1140 μg equivalent eprinomectin/kg in liver and 850 μg equivalent eprinomectin/kg in kidney.

In rats, whatever the dosage used, single administration of 15 mg/kg bw or repeated administration of 6 mg/kg bw/day for 7 days, the following components were identified in tissues : eprinomectin B1a, eprinomectin B1b, the metabolite M5 and the N-deacetylated metabolite of eprinomectin B1a.

5. After a topical application of 0.50 mg/kg bw radiolabelled eprinomectin (both homologues being radiolabelled) to 8-10 month old calves, only a small percent of the applied dose (0.35%) was found in the urine through 28 days and 17% to 19.8 % in the faeces. In faeces, the eprinomectin B1a was the most abundant residue and represented 78.3% of the total residues, eprinomectin B1b 8.3 %, and another compound, metabolite M1 7.4% of the total radioactivity, the 4 other metabolites identified M2, M3, M4, M5 being in very low amount (less than 1.6 %).

The amount of the drug excreted in milk represents 0.32 to 0.54 % of the radioactivity through day 14.

6. After a topical application of eprinomectin containing radiolabelled eprinomectin B1a to cows at a level dose of 0.750 mg/kg bw, the highest ^3H -residues and ^{14}C -residue levels in plasma were in the range 47-109 μg equivalent eprinomectin/l and were observed between 1-5 days. By day 21, the total residue amount was below 5 μg equivalent eprinomectin/l. The major metabolite was eprinomectin B1a, representing about 90 % of the total residues.

7. Eprinomectin was found to be highly bound (greater than 99%) to cattle plasma proteins for the following range of concentrations 7.5 to 213 µg/l, figures corresponding to the plasma concentrations after treatment at the recommended dosage.
8. The fraction of eprinomectin B1a absorbed after the application of a pour-on formulation was assessed by comparison of the area under the curve obtained after intravenous administration at different levels doses (0.025 mg, 0.050 and 0.100 mg eprinomectin/kg bw intravenously) to the area calculated after a single topical application (0.500 mg/kg bw). Only 29 % of the dose was absorbed through the skin. Most of the absorption occurred within 7-10 days post dose following an initial time-lag of about 24 h and continued to a minor extent 17-21 days post dose.

After a topical application of 0.500 mg/kg bw of radiolabelled eprinomectin, 54% of the radiolabelled dose remained on the hide. 89% of the total extractable radioactivity was the non metabolised parent compound.
9. The acute toxicity of eprinomectin was determined in mice and rats. In female mice, the oral and intraperitoneal DL₅₀ values were 70 and 35 mg/kg bw whereas in female rats the DL₅₀ values were 55 and 35 mg/kg bw after oral or intraperitoneal administrations respectively.
10. In a 14-week repeated toxicity study carried out in rats, eprinomectin was administered at level doses of 0, 1, 5 and 20 mg/kg bw/day. Animals treated with the highest dose showed ataxia, tail and body tremors and degeneration of the sciatic nerves was noted in 15 % of animals of both sex. A NOEL of 5 mg/kg bw/day was retained for this study.

In a 14-week repeated toxicity study carried out in dogs, eprinomectin was administered at level doses of 0, 0.4, 0.8 and 1.6 mg/kg bw/day. Animals treated with the highest dose showed mydriasis, ataxia, salivation. A NOEL of 0.8 mg/kg bw/day was retained for this study.
11. A fifty-three week oral toxicity study was conducted in dogs receiving eprinomectin at level doses of 0, 0.5, 1 and 2 mg/kg bw/day. At the highest level, mydriasis was reported. Histopathological examination showed a slight focal degeneration in the pons area and/or the cerebellar nuclei in 3 out of 8 dogs. This degenerative change was characterised by neuronal enlargement that resulted from increased eosinophilic vacuolated cytoplasm with nuclear displacement. Although this change affected 1 to 3 neurones/dog, it was attributed to treatment because other compounds in this class have caused neuronal degeneration in dogs in this area of the brain. 1 mg/kg bw/day was the NOEL.
12. In the tolerance studies, no drug-related abnormal clinical observations nor side-effects were observed in cattle after treatment at 1, 3 or 5 times the therapeutic dose, three times at 7-day intervals or by cows treated at 10 times the therapeutic dose.

No adverse effects on the quality of sperm and reproductive performance of bulls treated at 3 times the therapeutic dose were observed.

Eprinomectin was shown to be safe when topically administered at least three times the recommended dose level to breeding females throughout the reproductive cycle.
13. A two-generation reproduction study with 1 litter for the first generation and 2 litters for the second generation was performed in rats, eprinomectin being administered in feed at dose equivalent to 1, 2.5-3, and 6 mg/kg bw/day. Maternotoxicity characterised by a diminution of the mating/reproductive performance was observed at the highest level specially in adult F1. An increase in pup mortality, a marked reduction in pup growth and body tremors among all pups were reported at the highest level. Treatment-related body tremors were also noted at 2.5-3 mg/kg bw in 4 out of 26 litters of the F2A generation. So, 1 mg/kg bw/day was retained as the NOEL for growth and reproductive performance of the rat.

Investigations showed that there was a consistent milk-to-plasma concentration ratio of about 3:1 throughout the study, demonstrating that the concentrations of drug in rat milk resulted in enhanced neonatal exposure in nursing pups.
14. Teratogenicity studies were carried out in rats and rabbits.

In rats, eprinomectin was given in the feed at equivalent doses ranging from 0.4 to 14 mg/kg bw/day. No adverse effects on dams were observed for dosages up to 1 mg/kg bw/day and on pups for doses up to 14 mg/kg bw/day. No teratogenic effect was observed.

In three studies conducted in rabbits, eprinomectin was administered by gavage in rabbits at doses between 0.5 to 8 mg/kg bw/day. Mydriasis and slowed pupillary reflex to light in females were reported for the doses higher or equal to 2 mg/kg bw/day. The NOEL of eprinomectin in rabbits for the maternal toxicity was 1.2 mg/kg bw/day. No evidence of foetotoxicity or teratogenicity were noted up to 8 mg/kg bw/day.

15. In a set of mutagenicity tests [Ames test, *in vitro* gene mutation test with V-79 Chinese hamster lung cells at the HGRPT locus, *in vitro* chromosomal aberrations test (Chinese hamster ovary) and *in vivo* micronucleus assay in mice], eprinomectin did not show mutagenic activity. In addition, eprinomectin gave negative results in the *in vitro* alkaline elution/primary rat hepatocyte assay measuring DNA strand breaks.
16. No human data are available as eprinomectin has been developed exclusively for use in veterinary medicine.
17. No carcinogenicity studies were provided. As eprinomectin is not genotoxic and as this compound has no structural relationship to known carcinogens and as, in addition, closely related compounds showed no carcinogenic potential in long-term tests in rats and mice, the Committee concluded that carcinogenicity studies are not required for eprinomectin.
18. Based on the NOEL of 1.0 mg/kg bw/day from the 53-week toxicity study in dogs and retaining a safety factor of 200, a toxicological ADI of 0.005 mg/kg bw/day can be established.
19. The pharmacokinetic profile of eprinomectin in target species was well studied using radiolabelled compounds after pour-on applications of 0.750 mg/kg bw (2 studies) and of 0.500 mg/kg bw (one study).

After a single application of 0.50 mg/kg bw in the presence of labelled eprinomectin, both eprinomectin B1a and eprinomectin B1b being labelled, the nature of metabolites in edible tissues were determined. Several minor metabolites could be detected in edible tissues : the homologue eprinomectin B1b representing between 7.2 to 9.3 % of the total radioactivity, five to seven minor metabolites contributing in the range of 1-2% of the total residues excepted for muscle in which metabolite M5 accounting for 3.9% of the total residues.

However, the metabolism profile indicated that eprinomectin B1a was the major residue in all tissues and the following ratios marker residue towards total radioactivity were determined at 21 days withdrawal period : 75 % for muscle, 100 % for fat, 80 % for liver, 78 % for kidney.

The compound eprinomectin B1a was also the major metabolite accounting for 80-85.6% of the total extractable radioactivity in milk. The metabolite M1 represented less than 2%, M5 accounted 0.7-2.5% and the contribution by M2 and M4 was negligible (less than 1%).

20. In a first radiometric depletion study carried out in lactating cows, after a pour-on application of 0.750 mg/kg bw eprinomectin containing only $^3\text{H}/^{14}\text{C}$ component eprinomectin B1a, the total radioactive residue levels in tissues on day-21 post dose were : 0.11 µg equivalent eprinomectin/kg in muscle, 28.59 µg equivalent eprinomectin/kg in dose site muscle, 8.6 µg equivalent eprinomectin/kg in fat, 119.3 µg equivalent eprinomectin/kg in liver and 15.56 µg equivalent eprinomectin/kg in kidney.

In a second radiometric depletion study conducted on lactating cows receiving 0.750 mg/kg bw of eprinomectin containing radiolabelled components eprinomectin B1a and eprinomectin B1b, at 21 days post application, the following amounts of radioactivity were measured in edible tissues : 0.7 µg equivalent eprinomectin/kg in muscle, 87.5 µg equivalent eprinomectin/kg in the dose site muscle, 12.2 µg equivalent eprinomectin/kg in fat, 145.8 µg equivalent eprinomectin/kg in liver, 21.4 µg equivalent eprinomectin/kg in kidney.

21. In a radiometric depletion study carried out in cattle at the therapeutic dosage (0.50 mg/kg bw), the following concentrations of marker residue, eprinomectin B1a, were measured in edible tissues : 6 µg/kg in muscle, 17 µg/kg in dose-site muscle, 30 µg /kg in fat, 807 µg/kg in liver and 161 µg/kg in kidney, 7 days after application. Then the concentrations declined rapidly to reach 3 µg/kg in muscle, 14 µg/kg in dose-site muscle and in fat, 369 µg/kg in liver and 54 µg/kg in kidney, 21 days after application.

The half-lives for the depletion of the radioactivity in liver, kidney, fat and muscle were 8.6, 8.1, 7.9 and 7.8 days, respectively. In dose site muscle, the depletion half-life was longer (36.1 days).

22. In a non radiometric study carried out in cattle at the therapeutic dose (0.5 mg/kg bw), the concentrations of eprinomectin B1a were measured in all edible tissues. At 10 days, 6-8 µg/kg could be measured in muscle, 26 µg/kg in fat, 748 µg/kg in liver and 74 µg/kg in kidney. Then, these levels decrease to attain 2 µg/kg in muscle, 8 µg/kg in fat, 237 µg/kg in liver and 40 µg/kg in kidney at 17 days post-application. 44 days after the application, only traces of eprinomectin could be detected in liver.

23. Two radiometric studies with regard to the depletion of eprinomectin in milk were carried out in lactating cows after topical application of 0.750 mg/kg bw eprinomectin. In the first study, the levels of radioactivity in milk ranged from 1.45 to 5.36 µg/kg during the 20 days following the treatment. In the second study, they were below 10 µg equivalent eprinomectin/kg in nearly 91 % of the samples assayed during 21 days following the treatment. Very low levels of radioactivity (0.4-1.5 µg/kg) could be detected until 21 days post treatment.

24. In another radiometric study, eprinomectin was applied at a level dose of 0.750 mg/kg bw to six pregnant dairy cows 7-14 days prior the parturition. The highest radioactive residue levels in colostrum of the cows were 13.18 µg equivalent eprinomectin/kg on parturition days occurring at 7.7 days post dose (1 animal) and in the range 5.61-12.27 µg equivalent eprinomectin/kg, on parturition days when occurring at 14.5 days post dose (5 animals). At 7 days post-parturition, the radioactivity levels in milk ranged from 3.92 to 2.38 µg equivalent eprinomectin/kg.

The highest levels of eprinomectin B1a ranged from 1.32 to 7.88 µg/kg in colostrum. At 7 days post-parturition, the eprinomectin B1a concentrations were below 0.50 µg/kg.

25. In a non radiometric study, 20 dairy cows were dosed topically with 0.500-0.547 mg eprinomectin/kg bw. The mean highest concentration of eprinomectin B1a was close to 5 µg/kg with the peak occurring in 80% of the animals 5-6 milkings after treatment. Concentrations close to 0.5 µg/kg could still be detected at the 13th milking.

26. Validated analytical methods have been developed to measure residues of eprinomectin B1a, the marker residue of eprinomectin in edible tissues and in milk. For tissues, the limit of quantification is 2 µg/kg and the limit of detection 1 µg/kg. For milk, the limits of quantification and detection were 1 µg/kg and 0.25 µg/kg respectively.

27. Having considering that :

- a toxicological ADI of 0.005 mg/kg bw has been established;
- the metabolism of eprinomectin is limited;
- eprinomectin B1 is the marker residue,
- the ratio of parental compound towards total residues is known for all edible tissue including milk : 75 % for muscle, 100 % for fat, 80 % for liver, 78 % for kidney, 80-85 % for milk ;
- validated analytical methods are available for monitoring residues in edible tissues;

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

Pharmacologically active substance(s)	Marker residue	Animal species	MRL	Target tissue	Other provisions
Eprinomectin	Eprinomectin B1a	Bovine	30 µg/kg 30 µg/kg 600 µg/kg 100 µg/kg 30 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRLs values, the daily intake will represent about 50 % of the toxicological ADI.