The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and Information Technology

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NETOBIMIN

SUMMARY REPORT (2)

1. Netobimin is a carbamate compound which is used as an anthelmintic drug in veterinary medicine. Netobimin-containing products are available as liquid suspensions which are intended for oral administration. Products are available for sheep and cattle. An oral dose of 7.5 mg/kg bw is recommended for the treatment of gastrointestinal infestations of roundworms and tapeworms; and 20 mg/kg bw is recommended for type II ostertagiasis and adult flukes. Products for parenteral use are no longer marketed.

   Provisional MRLs have already been established for netobimin with the marker residue defined as the sum of netobimin and albendazole and metabolites of albendazole measured as 2-amino-benzimidazole sulphone.

   Currently, netobimin is included 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
Netobimin	Sum of netobimin and albendazole and metabolites of albendazole measured as 2-amino-benzimidazole sulphone	Bovine, ovine, caprine	100 µg/kg 100 µg/kg 1000 µg/kg 500 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	Provisional MRLs expire on 31.07.1999

   Additional data in support of the establishment of final MRLs concerning bovine and ovine species only and restricted to oral administration have now been submitted.

2. In order to be pharmacologically active, netobimin needs to be converted to the broad spectrum anthelmintic drug albendazole, by the splitting off of a side-chain and the formation of a benzimidazole group. This occurs naturally in the ruminant gut.

3. When netobimin was given to female non-pregnant rats at a single oral dose of 59.5 mg/kg bw, albendazole was detectable in the serum at 6 hours post-dosing, and for serum albendazole oxide and serum albendazole sulphone respectively, the C_{max} values were 1.6 and 0.2 µg/ml; the t_{max} values were 6 and 12 hours, the half-lives were 0.14 and 5.1 hours; and the area under the curve values were 32.7 and 7.5 µg.hr/ml. When pregnant rats were given the same oral dose, the area under the curve values were 15.5 and 2.6 µg.hr/ml for albendazole oxide and albendazole sulphone respectively. When oral netobimin doses of 50.0, 59.5 or 70.7 mg/kg bw were given to pregnant rats, albendazole, albendazole oxide and albendazole sulphone crossed the placenta to be detected in the amniotic sacs and embryos. The mean concentrations of albendazole, albendazole oxide and albendazole sulphone in the embryos were all higher than the concurrent plasma levels. In serum, amniotic sacs and embryos, the concentrations of albendazole oxide were higher than those of albendazole sulphone which were higher than albendazole.

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4. Albendazole was readily absorbed from the ruminant gut (cattle absorb about 50% of oral doses of albendazole). The degree of absorption of unchanged netobimin was minimal, and only traces have been detected in tissues and milk from orally-treated ruminants. Following absorption, the albendazole underwent subsequent metabolism. The principal route for the primary metabolism of albendazole in farm animals and in laboratory animals was by rapid first pass oxidation of its sulphide group to form a sulphoxide (albendazole oxide), then further oxidation to form a sulphone (albendazole sulphone), and by deacetylation of the carbamate group to form an amine. Albendazole, albendazole oxide, albendazole sulphone and albendazole-2-aminosulphone were the main metabolites found in tissues irrespective of whether the animals were dosed with netobimin, albendazole or albendazole oxide. Other metabolites have been detected only at much lower concentrations. No information was available on the metabolic fate of the side chain ($NHCH_2CH_2SO_3H$) which split off from the netobimin molecule when albendazole was formed. Excretion of orally administered netobimin is via urine and faeces: in adult cattle and sheep the percentage of the administered dose which was excreted in urine was 45% (cattle) and 48% (sheep) with the percentages in faeces being 37% (cattle) and 40% (sheep). However, in calves the percentage excreted in urine (31%) was less than that in faeces (46%).

5. After subcutaneous or intramuscular injection of netobimin into cattle, absorption was rapid (t_{max} 0.1 to 1.5 hours) but plasma levels of radioactivity were lower than those achieved after oral administration (equivalent to 2 µg/ml for subcutaneous injection, 4.23 µg/ml for intramuscular injection and 5.92 µg/ml for oral administration). Absorption occurred prior to conversion to albendazole since high levels of parent compound occurred in plasma and milk but by 12 hours after injection, no parent compound was found at the injection site or in liver.

6. Since much of the administered netobimin was converted in the target animal to albendazole, safety data from the testing of albendazole and its metabolites (including albendazole oxide) were considered to be relevant to the safety assessment of netobimin. However, it was recognised that netobimin may have additional toxicological properties of its own.

7. Experience from use of albendazole in human medicine shows that oral doses of albendazole are not well absorbed from the human gut: about 1% is absorbed. Thus oral exposure to food residues of albendazole resulting from the veterinary use of netobimin would be expected to be less toxic to humans than to laboratory animals or farm animals.

8. Netobimin was not tested for irritancy, but albendazole oxide was not irritant to the skin or eyes of rabbits.

9. No information on the sensitising potential of netobimin was available. However, a positive result in a guinea-pig maximisation test showed albendazole oxide to be a potential skin sensitiser.

10. Netobimin, albendazole and albendazole oxide were all of low acute toxicity when given by the oral route or parenteral routes. No deaths were caused with oral doses of up to 6900 mg/kg bw in mice, 5000 mg/kg bw in rats and 2000 mg/kg bw in dogs. Intravenous LD_{50} values were 344 and 570 mg/kg bw in male and female mice, respectively, and 786 and 659 mg/kg bw for male and female rats, respectively.

11. In repeated oral dose studies, the toxicological profiles of netobimin, albendazole and albendazole oxide were very similar, with one exception. Skin lesions (ulcerated or abscessed skin with secondary systemic infections) were produced in rats by high doses of netobimin, but not with albendazole or albendazole oxide. This may have been due to toxicity of the side chain which is broken off the netobimin molecule when it is metabolised to albendazole. All three substances caused hepatotoxicity and testicular toxicity, with albendazole causing these effects with an NOEL of 7 mg/kg bw/day and albendazole oxide having an NOEL of 10.9 mg/kg bw/day for both effects. Netobimin only produced testicular toxicity at higher doses with an NOEL for this effect of 60 mg/kg bw/day, but no NOEL was established for the hepatotoxicity of netobimin. Vacuolation of the hepatocytes was seen at the lowest dose used: 15 mg/kg bw/day (from a one year rat study).

12. Developmental studies in mice, rats, rabbits and sheep showed all three compounds to be teratogens with similar potencies. The malformations included visceral, craniofacial and bone defects (including shortened limbs). The lowest NOEL for any of the studies was 5 mg/kg bw/day for albendazole administered orally to rats. The NOEL for developmental effects following dosing with netobimin was 7.5 mg/kg bw/day.

13. Reproductive effects of netobimin and albendazole were investigated in multigeneration oral-dosing studies in rats. Albendazole caused reduced survival and growth of pups during the post-natal lactation period (NOEL: 5.8 mg/kg bw/day), but netobimin caused decreased pup growth only at doses of 42 mg/kg bw/day or more (no effects at 15 mg/kg bw/day).

14. Netobimin produced negative results in bacterial mutation tests (*Salmonella* microsomal assay in TA1535, TA1538, TA97, TA98 and TA100 with and without S9). It was not mutagenic in a mouse lymphoma assay. Netobimin was also negative in an *in vitro* UDS assay and in an SOS Chromotest of DNA repair. On the other hand, netobimin was positive in an *in vivo* mouse bone marrow micronucleus test. Albendazole and albendazole oxide were also positive in *in vivo* mouse bone marrow micronucleus tests. Albendazole oxide also gave a positive result in a yeast assay for aneugenicity. These results of mutagenicity testing of netobimin, albendazole and albendazole oxide were consistent with them being *in vivo* somatic cell mutagens. In the absence of any tests on germ cells, it remains unclear whether or not these substances can induce heritable mutations. It was considered likely that the mechanism of the mutagenicity of these substances involved the induction of aneugenicity as a result of inhibition of the mitotic spindle.

15. Netobimin has not been tested in any long-term studies nor carcinogenicity bioassays, but albendazole has been adequately tested in carcinogenicity bioassays, giving no evidence of neoplasia in either rats or mice. Although netobimin produced no carcinogenicity in a one-year rat feeding study, this was regarded as being of insufficiently long duration for the investigation of carcinogenicity.

16. The toxicological profile of orally-administered netobimin was similar to that of albendazole and albendazole oxide, but some additional toxic effects were observed. In particular, it caused skin lesions in addition to the effects observed with the other two drugs. NOELs of 30 and 60 mg netobimin/kg bw/day were identified for the skin lesions in female and male rats, respectively. As the oral administration of netobimin did not result in any residues of parent drug in edible tissues or milk, the ADI of 0.005 mg/kg bw which was set for albendazole and albendazole oxide may be used as the basis for setting MRLs for netobimin. This ADI was set on the basis of an NOEL of 5 mg/kg bw/day for the teratogenicity of albendazole in rats and rabbits using a safety factor of 1000. Parenteral uses of netobimin are contra-indicated because unchanged netobimin may form a part of the residue.

17. Radiolabel studies were carried out using netobimin uniformly labelled with ^{14}C in the benzene ring. In calves given a single oral dose of netobimin at 20 mg/kg bw, total residues were highest and most persistent in liver (greater than 22 000 µg/kg) at 10 hours, depleting to around 3500 µg/kg 10 days after treatment. Levels in kidney were also around 22 000 µg/kg at 10 hours, but depleted more rapidly, having reached 620 µg/kg by 10 days. Residues in muscle and fat were much lower at all time points and depleted to 43 µg/kg (muscle) and 97 µg/kg (fat) by 10 days. Levels in adult cattle receiving the same dose were rather higher. Total residues in milk were greater than 5000 µg/kg at 8 hours after treatment, reducing to 60 µg/kg after 71 hours.

    When sheep were given a similar oral dose of netobimin, total residues were generally lower than those in cattle, e.g. by 10 days after treatment, levels were 1340 µg/kg in liver, 303 µg/kg in kidney, 57 µg/kg in muscle and 10 µg/kg in fat. Experimental data on residues in sheep's milk indicate that total residues are rather higher than in cows' milk.

18. Final residue depletion studies were conducted using non-radiolabelled netobimin in cattle and sheep using 3 animals per time point and an oral dose of 20 mg/kg bw. Residues were measured by HPLC and the results, when compared with those from the radiolabel studies, indicated that residues comprising albendazole, albendazole oxide, albendazole sulphone and albendazole 2-aminosulphone accounted for nearly all of the residue in muscle and fat at time points ranging from 18 hours to 20 days. However, in liver and kidney these metabolites accounted for less of the total residue as time progressed, indicating the presence of bound residues.

19. A single oral dose of 20 mg/kg bw netobimin was administered to male and female cattle. The animals were slaughtered (3 per time-point) at 10 hours, 3 days, 7 days and 14 days after dosing. Residues of albendazole were detected only at the first time point (mean residues were 7354 µg/kg in liver, 3285 µg/kg in kidney, 792 µg/kg in muscle and 556 µg/kg in fat).

    No residues of albendazole 2-aminosulphone were detectable in liver at 10 hours but mean residues of 243 µg/kg were found at 3 days; these residues depleted to 213 µg/kg at 7 days and 81 µg/kg at 14 days; no residues of albendazole 2-aminosulphone were detectable in other tissues.

    At 10 hours, mean residues of albendazole oxide were 771 µg/kg in liver, 467 µg/kg in kidney, 1909 µg/kg in muscle and 195 µg/kg in fat and were undetectable in these tissues at later time points. At 10 hours, mean residues of albendazole sulphone were 5289 µg/kg in liver, 1879 µg/kg in kidney, 1740 µg/kg in muscle and 474 µg/kg in fat and were undetectable in these tissues at later time points.

20. A single oral dose of 20 mg/kg bw netobimin was administered to male and female sheep. The animals were slaughtered (3 per time-point) at 18 hours, 3 days, 10 days, 20 days and 30 days after dosing. At 18 hours, mean residues of albendazole were 7400 µg/kg in kidney, 2200 µg/kg in muscle and 303 µg/kg in fat and were undetectable in these tissues at later time points. In liver, mean residues of albendazole were 18 000 µg/kg at 18 hours and low residues of albendazole persisted in liver up to the end of the study (9 to 12 µg/kg over the period 10 to 30 days.

    Mean residues of albendazole 2-aminosulphone were 78 µg/kg in liver at 18 hours, 770 µg/kg at 3 days and 150 µg/kg at 10 days. Mean residues of albendazole 2-aminosulphone were 110 µg/kg in kidney at 18 hours, 226 µg/kg at 3 days and 31 µg/kg at 10 days. Low residues of albendazole 2-aminosulphone were found in muscle for up to 10 days after dosing and in fat for up to 3 days after dosing.

    At 18 hours, mean residues of albendazole oxide were 3100 µg/kg in liver, 2400 µg/kg in kidney, 3400 µg/kg in muscle and 983 µg/kg in fat; residues of this metabolite were detectable at 3 days in kidney (150 µg/kg) and up to 20 days in liver but were undetectable in muscle and fat beyond the first time point. At 18 hours, mean residues of albendazole sulphone were 4200 µg/kg in liver, 110 µg/kg in kidney, 1100 µg/kg in muscle and 555 µg/kg in fat; low residues of this metabolite were detectable for up to 3 days in liver and 10 days in kidney but were undetectable in muscle and fat beyond the first time point.

21. In milk samples from cattle and sheep, residues of netobimin and albendazole were undetectable. No 2-aminosulphone was detected at any time point, presumably because total residues had depleted to less than 100 µg/kg by 84 hours (3.5 days) after treatment and relatively little 2-aminosulphone had been formed before this time. In sheep dosed orally with 20 mg/kg bw netobimin, milk samples collected at 8.5 hours after dosing consisted of 93.7% albendazole oxide and 6.4% albendazole sulphone and milk samples collected over the period 8.5 to 23 hours after dosing consisted of 35% albendazole oxide and 64% albendazole sulphone. Following oral administration of 20 mg/kg bw netobimin to 5 lactating cattle, mean residues of albendazole oxide in milk depleted from 7374 µg/kg at 7.5 hours to 2 278 µg/kg at 22 hours and 210 µg/kg at 31.5 hours; mean residues of albendazole sulphone were 2336 µg/kg at 7.5 hours, 3 410 µg/kg at 22 hours and approximately 80 µg/kg at 31.5 hours.

22. Following the administration of netobimin to the target species, detectable residues of albendazole in tissues were normally present for only a few hours after dosing. For this reason, and also to be consistent with the recommendations for albendazole and for albendazole oxide, it was agreed that the same marker residue should apply for all three substances, i.e. the sum of albendazole oxide plus albendazole sulphone plus albendazole 2-aminosulphone. It was considered that this marker residue would account for all of the residues of toxicological significance in milk at all time points and almost all of the residues of toxicological significance in tissues from 3 days after dosing.

23. A validated analytical method for the determination of residues of albendazole, albendazole oxide, albendazole sulphone and albendazole 2-aminosulphone in cattle and sheep tissues including milk was provided. The method involved solvent extraction, acid hydrolysis and solid phase clean-up prior to quantification by HPLC-MS/MS. The limit of quantification (expressed as sum of the metabolites) was 500 µg/kg for liver, 250 µg/kg for kidney and 50 µg/kg for muscle, fat and milk.

Conclusions and recommendation

Considering that:

- netobimin is a pro-drug of albendazole and its pharmacological activity depends on its conversion to albendazole,
- netobimin is not administered parenterally and oral administration does not result in any residues of parent drug in edible tissues or milk, the ADI of 0.005 mg/kg bw (i.e. 0.3 mg/person) which was established for albendazole and albendazole oxide may be used as the basis for setting MRLs for netobimin,
- the principal components of the extractable residue in cattle and sheep treated orally with netobimin were albendazole oxide, albendazole sulphone and albendazole 2-aminosulphone; residues of albendazole were not normally present in tissues at time points from 3 days after dosing,
- the residues in milk from cattle and sheep, consisted almost entirely of albendazole oxide and albendazole sulphone at all time points,
- for the sake of consistency, it was considered that the choice of marker residue and the numerical values of the MRLs should be the same as established for albendazole and albendazole oxide,
- a validated analytical method is available for the determination of albendazole oxide, albendazole sulphone and albendazole 2-aminosulphone;

the Committee recommends the inclusion of netobimin in Annex I of Council Regulation (EEC) 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Netobimin	Sum of albendazole oxide, albendazole sulphone and albendazole 2-aminosulphone, expressed as albendazole	Bovine, ovine	100 µg/kg 100 µg/kg 1000 µg/kg 500 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	For oral use only

Based on these MRLs, it was calculated that the daily intake of extractable residues would amount to 310 µg/day, i.e. 103% of the ADI. It was considered that this would not constitute a risk to consumers because at least 75% of the residues in tissues were not bioavailable.