European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

MORANTEL
(Extension to all ruminants)

SUMMARY REPORT (3)

1. Morantel (1,4,5,6-tetrahydro-1-methyl-2-[2-(3-methyl-2-thienyl)ethenyl pyrimidine) is a tetrahydro-pyrimidine anthelmintic, differing from the related analogue pyrantel by the presence of a methyl group on the thiophene ring. It is intended to treat roundworms and tapeworms. It is administered in lactating and non-lactating cattle as morantel tartrate as a slow-release bolus (11.8 g morantel base per animal) or as a single oral dose of 6 to 7.5 mg morantel base/kg bw and in pigs at a single dose equivalent to 7.5 mg base/kg bw. In sheep, the citrate salt is administered at a single dose equivalent to 5 to 6 mg morantel base/kg bw.

   Morantel and its salts are not used in human medicine.

   Currently, morantel 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
Morantel	Sum of residues which may be hydrolysed to N-methyl-1,3-propanediamine and expressed as morantel equivalents	Bovine, ovine	100 µg/kg 100 µg/kg 800 µg/kg 200 µg/kg 50 µg/kg	Muscle Fat Liver Kidney Milk	

2. A request was submitted to the EMEA for the extension of the existing entry in Annex I of Council Regulation (EEC) No. 2377/90 to all ruminant species. The scientific justification for this extension was assessed taking into account the Note for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187-00-FINAL).

3. In setting the ADI in the original assessment of morantel, the data summarised on the paragraphs below were considered.

4. Morantel acts as a potent agonist at the acetylcholine receptors on the muscle cells of nematodes. Activation of the acetylcholine receptors induces a prolonged, spastic paralysis of the worms and expulsion from the host. It also been reported to block neurotransmission in vertebrates, to possess nicotine-like properties and to mimic acetylcholine at receptors in autonomic ganglia, adrenal medullae and respiratory tissues.

5. In mice, after single oral administrations of 3H- or non-radiolabelled morantel citrate at doses equivalent to 50 mg morantel base/kg bw or of 3H-morantel tartrate at doses equivalent to 6 mg morantel base/kg bw, approximately 27% of the administered dose were excreted in urine within 24 hours. A large number of metabolites were detected but non-identified. The parent compound represented only 2.6% of the dose administered in urine. After a single oral administrations of radiolabelled morantel citrate at doses equivalent to 50 mg morantel base/kg bw, the mean plasma concentrations of morantel were 4.8 and 3.7 µg equivalents/ml, 1 and 2 hours after the administration. No parent compound was detected in plasma after 24 hours. After the last of 3 oral administrations of morantel citrate at doses equivalent to 50 mg morantel base/kg bw, the highest plasma concentration of the parent compound (1.06 µg/ml) was observed 1 hour after administration and its elimination half-life was 1.7 hours.

   After single oral administrations of morantel tartrate at doses equivalent to 6 and 30 mg morantel base/kg bw in rats and in dogs, 8% and 43% of the administered dose were excreted in urine within 24 hours, respectively.

   In bovine, after a single oral administration ^{3}H- or ^{14}C-morantel tartrate at a dose equivalent to 6 mg morantel base/kg bw, less than 20% (13%) of the administered dose was recovered in urine over 96 hours, the remaining part being excreted via faeces (69%).

   In sheep, after single oral administrations of ^{14}C-morantel citrate at a dose equivalent to 5 to 6 mg morantel base/kg bw, 18% and 67% of the radioactivity administered was excreted in urine and faeces within 4 days, respectively.

   In all species, a substantial proportion of the administered dose was excreted in the faeces as unmetabolised morantel. Morantel is metabolised by three metabolic pathways (oxidation of the thiophene ring, oxidation of the tetrapyrimidine ring and conjugation with glutathione). The oxidative metabolism of the thiophene ring of the urinary radioactivity leads to acidic metabolites, which are highly polar (4-ketohept-2-eneldioic acid, levulinic acid, 4-ketopimelic acid and α-ketoglutaric acid). This acid fraction represents 3% (sheep) to 25.7% (dogs) of the urinary radioactivity. Around 57% (dog) to 86% (rats) of the urinary radioactivity, convertible to N-methyl-1,3-propanediamine, were issued from the metabolites containing the tetrahydropyrimidine ring.

   In vitro data, on liver microsomes of rat, dog and cow confirmed these biotransformations leading to the formation of the same 8 metabolites already identified *in vivo* studies.

6. The oral LD_{50} values for morantel tartrate, were equivalent to 179 to 260 and 551 to 586 mg morantel base/kg bw in mice and rats, respectively. For the citrate salt, the oral LD_{50} values were in the magnitude of 125 mg morantel base/kg bw in mice. The main symptoms of intoxication were respiratory effects, hypothermia, ataxia, tremors and convulsions.

7. Two oral 3-month toxicity studies were conducted in rats.

   In the first study, animals received morantel tartrate in their diet at doses approximately equivalent to 0, 30, 90 or 270 mg morantel base/kg bw/day. In males, mortality was noted in the 270 and 90 mg/kg bw dose groups (40 and 30%, respectively) and histopathological findings (renal tubular necrosis and testicular atrophy) were recorded in the highest dose group. Haematological and biochemical changes were noted in the two highest dose groups and signs of haemorrhages were observed at doses equal to and higher than 30 mg/kg bw/day.

   In the second study, rats received morantel tartrate in their diet at doses equivalent to 0, 6, 12 and 30 mg morantel base/kg bw/day. No adverse effects were reported in this study.

   From these two studies, an overall NOEL of 12 mg/kg bw/day morantel base was retained.

8. In a 2-year toxicity study carried out in dogs, groups of 8 to 10 animals received morantel tartrate in gelatine capsules at doses equivalent to 0, 1.2, 6 and 12 mg morantel base/kg bw/day. Vomiting was frequently noted in the two highest dose groups was probably related to morantel’s parasympathomimetic activity. In the highest dose group, the absolute and relative liver and adrenal weights were significantly increased. A NOEL of 1.2 mg/kg bw/day morantel base was retained.

9. Morantel tartrate was well tolerated by oral route in sheep and bovine at doses of up to 6.5, 28 and 28 times the recommended doses, respectively.

10. No effects on male or female fertility were reported in the 3-generation study carried out in rats after oral administration of morantel tartrate at doses equivalent to 0, 0.6, 1.5 and 3 mg morantel base/kg bw/day. No statistically significant effects and no dose-related pup mortality were seen when compared to the control group. However, the conclusion regarding *peri*- or *post*-natal effects should be taken with caution due to the very high pup mortality (approximately 50%) in all dose groups including the controls.

11. Teratogenicity studies were carried out in rats, in rabbits and in mice.

    No evidence of teratogenicity, foetotoxicity or maternal toxicity was observed in rats after oral administration of morantel citrate or tartrate salts, up to doses equivalent to 50 to 60 mg base/kg bw/day. No statistically significant effects and no dose-related incidence of malformations were seen when compared to the control group.

    No evidence of teratogenicity, foetotoxicity or maternal toxicity was observed in a teratology study in the rabbit after oral administration of morantel tartrate up to a dose equivalent to 60 mg morantel base/kg bw/day.

    No evidence of teratogenicity, foetotoxicity or maternal toxicity was observed in mice after oral administration of morantel tartrate up to a dose equivalent to 60 mg morantel base/kg bw/day.

12. Five *in vitro* and one *in vivo* mutagenic assays were carried out with morantel citrate or tartrate. Although the results did not give any cause for concern, four of the *in vitro* studies were inadequate due to the lack of replication, absence of information on the storage of the test materials and omission of positive controls in some of the studies. Only the *in vitro* mouse lymphoma assay (7 concentrations in the range of 390 to 2205 µg morantel base/ml as tartrate) and the *in vivo* cytogenetics assay (mouse micronucleus test) after oral administrations of morantel citrate at doses equivalent to 2.8, 25.5 and 50 mg base/kg bw were regarded as adequate and giving negative results. Morantel can be considered as a non-mutagenic compound.

13. In a carcinogenicity study carried out in the rat, the animals received morantel tartrate in their diet at doses of 0, 1.2, 12 and 30 mg morantel base/kg bw/day for 104 weeks. The group sizes were inadequate for the proper evaluation of the carcinogenicity. A dose-related decrease in the absolute heart weights was noted in the females of the two highest dose groups. No significant dose-related trend in tumour incidence was noted and the absence of any structural alerts in the chemical structure of morantel and the lack of a mutagenic response indicated that further carcinogenicity studies were not warranted.

14. A toxicological ADI of 12 µg/kg bw (i.e. 720 µg/person) was established, based on the NOEL of 1.2 mg morantel base/kg bw from the 2-year study carried out in dogs and applying a safety factor of 100.

15. For the extension to include all ruminant species in Annex I the information summarised in the paragraphs below was taken into account.

16. Morantel is extensively metabolised *in vivo*. After 24 hours total residues contained around 50% polar and pharmacological inactive metabolites after cleavage of the ring and gluthation conjugation. All the residues of morantel and its major metabolites can be converted following alkaline hydrolysis to N-methyl-1,3-propanediamine which is assayed by gas or liquid chromatographic methods. They can be also hydrolysed in presence of hydrochloric acid to 3-(3-3-methyl-2-thienyl) acrylic acid, which is assayed by a liquid chromatographic method.

17. In bovines, two radiometric studies after single administrations and two non-radiometric studies after intraruminal administration of boluses were initially provided.

In a first radiometric study, calves (6 to 8 weeks, 50 to 60 kg) received a single oral dose of ^{14}C-morantel tartrate at a dose of 5.9 mg morantel base/kg bw. Groups of 1 to 3 animals were slaughtered at 7, 14 and 28 days after treatment. Seven days after the administration, only levels of radioactivity (morantel equivalents) were measured: 60 µg/kg for kidney, 20 µg/kg for fat, and concentrations below 10 µg/kg (limit of quantification) for muscle. For liver, the amounts of radioactivity measured were 495, 250 and 140 µg/kg, at 7, 14 and 28 days post-dose, respectively.

After hydrolysis, residues in liver related to morantel were converted into N-methyl-1,3-propane diamine and the ratios of this compound towards total residues were as follows: 59% (n=2), 54% (n=1) and 40% (n=2) at 7, 14 and 28 days post-administration, respectively. No information was provided for the other edible tissues.

In a second radiometric study, 5 Holstein dairy cows (weight not stated) received a single oral dose of ^{3}H-morantel citrate at a dose equivalent to 5 mg morantel base/kg bw, in gelatine capsule. Four days after administration, total drug-related residues in liver averaged 1150 µg/kg. About half of the radioactivity was unextractable. However, in absence of information on the comparison of this value to the amounts of morantel related residues convertible to N-methyl-1,3-propanediamine or to 3-(3-3-methyl-2-thienyl) acrylic acid, no further consideration can be given to this information.

In one non-radiometric depletion study of morantel in edible tissues of bovine, morantel tartrate as sustained release formulation over a period of 90 days (12 g morantel base/bolus/animal) was administered by intraruminal route to calves. Five animals were slaughtered at each time point, i.e. 1, 15, 30, 45, 60, 75, 90 and 120 days following the administration. The residues of morantel were analysed after being converted to N-methyl-1,3-propanediamine (method not clearly indicated). For the complete time range the concentrations calculated as morantel base equivalents ranged from 150 to 300 µg/kg in liver. On days 45 and 90 the residue concentrations in muscle were close to 100 µg/kg whereas for kidney, they were 200 µg/kg.

An additional tissue residue depletion study was conducted in cattle following oral administration of [^{14}C]-morantel tartrate at single dose equivalent to 6 mg morantel base/kg bw. The plasma pharmacokinetics, the excretion and the metabolic profiling were documented. The residue depletion study was realised with 20 animals. Four animals were slaughtered at each sampling time: 1, 4, 7, 10 and 14 days following single oral administration at 6 mg morantel base/kg bw. Mean total radioactive residues declined from 31 to 11 µg/kg, 3008 to 412 µg/kg, 1145 to 76 µg/kg and 134 to 12 µg/kg in muscle, liver, kidney and fat between day 1 and day 14. The morantel marker residue: N-methyl-1,3-propanediamine, was analysed by GC-MS. This study permitted to estimate the ratio of marker to total residue for all edible tissues. Four days following treatment, the maximum marker residue observed levels were 28, 1149 and 195 µg/kg in muscle, liver and kidney, respectively. The ratios are 0.55, 0.40 and 0.35 in muscle, liver and kidney, respectively. In fat, the residue marker levels were below the limit of quantification.

Ruminating calves received an intraruminal bolus of morantel tartrate (corresponding to 12 g morantel base/bolus/animal). The concentrations of morantel residues, convertible into 3-(3-3-methyl-2-thienyl) acrylic acid were determined by an HPLC method in edible tissues collected on animals slaughtered (n=2 per time point) at 1, 2, 3, 5 and 7 days after the administration. At one-day post application, the concentrations of morantel related residues, expressed as morantel base equivalents were close to 15, 90 and 390 µg/kg in muscle, kidney and liver. Then, they declined slowly to reach 15, 40 and 150 µg/kg at 7 days after administration. No information is available for the other edible tissues.

Following a multiple dose regimen to simulate administration of the continuous release bolus, ^{14}C-morantel was administered orally to cattle for 10 or 20 day at a daily dose of 150 mg morantel base (19 or twice daily doses). Mean total residues in liver were 1702 µg/kg after 19 consecutive doses and 2190 µg/kg after 39 consecutive doses. In kidney, total residues were 371 µg/kg after 19 consecutive doses and 476 µg/kg after 39 consecutive doses. Residues in muscle were only 26 µg/kg and 24 µg/kg after 19 and 39 doses, respectively. In fat samples, residues were less than 45 µg/kg for all animals in both consecutive dose groups. The results did not indicate significant accumulation of total residues in tissues. For the multiple dose regimen, ratios marker/total residues were estimated to be 0.65, 0.49, 0.33 in muscle, liver and kidney, respectively.

18. One-radiometric and one non-radiometric depletion studies were conducted on sheep. Results from an old non-radiometric study were reported but it was considered inadequate due to the deficiencies of the analytical method (colorimetric method) and the low number of animal per slaughtering point.

Two animals received a single oral administration of ^{14}C-morantel tartrate at a dose equivalent to 9 mg morantel base/kg bw. At 7 days after treatment, the total radioactivity levels 20, 1130, 190, 20 µg morantel equivalents/kg in muscle, liver, kidney and fat, respectively. At 14 days, radioactivity levels were still high in liver (1050 µg/kg) and in kidney (80 µg/kg). In liver, the ratio of N-methyl-1,3-propanediamine/total residues was close to 60% at 7 and 4 days. No information on this ratio for the other edible tissues was available.

In a non radiometric depletion study in edible tissues of sheep, groups of 5 or 8 animals received a single oral administration of morantel citrate at a dose equivalent to 5 mg morantel base/kg bw. Residues in tissues were determined by gas chromatography after conversion of residues to N-methyl-1,3-propadiamine. At 3 days after treatment, the concentrations calculated as morantel base equivalent were less than 100, 985, 200 µg/kg in muscle, liver and kidney, respectively. After 7 and 14 days, no morantel (below 100 µg/kg) could be detected in muscle and kidney. However, for liver they declined to reach 402 and 240 µg/kg at 7 and 14 days after treatment. No data for fat were available.

An additional tissue residue depletion study was conducted in sheep following oral administration of [^{14}C]-morantel citrate at single dose equivalent to 6 mg morantel base/kg bw. The plasma pharmacokinetics, the excretion and the metabolic profiling were documented. The residue depletion study was realised with 20 animals. Four animals were slaughtered at each sampling time: 1, 4, 7, 10 and 14 days following single oral administration at 5.94 mg morantel base/kg bw. Mean total radioactive residues declined from 97 to 19 µg/kg, 5869 to 671 µg/kg, 1434 to 96 µg/kg and 34 to 6 µg/kg in muscle, liver, kidney and fat between day 1 and day 14. The morantel marker residue: N-methyl-1,3-propanediamine, was analysed by GC-MS. This study permitted to estimate the marker/total residue ratios for all edible tissues. Four days following treatment, the maximum residue marker observed levels were 36, 1234 and 263 µg/kg in muscle, liver and kidney, respectively. The ratios are 1, 0.51, 0.38 in muscle, liver and kidney, respectively. In fat, the residue marker levels were below the limit of quantification at all sampling times.

19. In a radiometric study, 5 Holstein dairy cows received a single oral administration dose of ^{3}H-morantel citrate at a dose equivalent to 5 mg morantel base/kg bw. In milk, the total radioactivity milk peaked at 84 µg/kg by the 2nd milking, then declined to reach 49 and 19 µg/kg by the 4th and the 6th milking, respectively. Morantel related residues convertible into N-methyl-1,3-propanediamine showed a parallel decline. The ratio of N-methyl-1,3-propanediamine/total residues averaged approximately 35% for all milkings.

Four non-radiometric depletion studies of morantel in milk were carried out in lactating cows.

In the first study, 11 lactating dairy cows received a single oral administration of morantel tartrate at a dose equivalent to 5.5 mg morantel base. The concentrations of morantel related residues were calculated either as N-methyl-1,3-propanediamine or as 3-[3-methyl-2-thienyl] acrylic acid. The peak time occurred at the second milking after administration with levels averaging 17 and 2.7 µg/kg for residues convertible to N-methyl-1,3-propanediamine and 3-[3-methyl-2-thienyl] acrylic acid, respectively. From the fourth milking, the mean levels were 10 and 1.6 µg/kg, respectively. This study showed that the fraction of morantel of residues converted into 3-[3-methyl-2-thienyl] acrylic acid is about ten times lower that the fraction converted into N-methyl-1,3-propanediamine.

For the three other studies, when morantel was administered as intraruminal boluses at the therapeutic regimen, it was shown that the concentrations of morantel related residues calculated either as N-methyl-1,3-propanediamine or as 3-[3-methyl-2-thienyl] acrylic acid were always below 100 µg/kg.

A new milk residue depletion study was realised in cattle following oral administration of ^{14}C-morantel tartrate at single dose equivalent to 6 mg morantel base/kg bw. The residue depletion study was realised with 8 lactating dairy cows. Milk was sampling twice daily for 7 days following single oral administration at 6 mg morantel base/kg bw. In milk, the mean total radioactivity peaked at 61 µg/kg by the 2nd milking (24h), then declined to reach 34 and 12 µg/kg by the 4th (48h) and the 6th (72h) milking, respectively. The morantel marker residue: N-methyl-1,3-propanediamine, was analysed by GC-MS. This study permitted to estimate the marker/total residue ratios for bovine milk. Only 2 samples at 24 hours were above the limit of quantification. The highest concentration of residue marker was 20 µg/kg. The ratio was 0.24.

20. In a depletion study of morantel in milk conducted in sheep, residues of morantel related residues calculated as N-methyl-1,3-propanediamine were always below 60 µg/kg after the administration of morantel tartrate at a single oral dose equivalent to 6 mg morantel base/kg bw.

A new milk residue depletion study was realised with 8 lactating dairy sheep following oral administration of ^{14}C-morantel tartrate at single dose equivalent to 6 mg morantel base/kg bw. Milk was sampled twice daily for 7 days following single oral administration at 6 mg morantel base/kg bw. In milk, mean total radioactivity peaked at 54 µg/kg by the 2nd milking (24h), then declined to reach 28 and 12 µg/kg by the 4th (48h) and the 6th (72h) milking, respectively. The morantel marker residue: N-methyl-1,3-propanediamine, was analysed by GC-MS. This study permitted to estimate the ratio of marker to total residue for bovine milk 24 hours following treatment, the maximum residue marker observed levels were 38 µg/kg in milk. The ratio was 0.44.

21. Based on data from radiometric studies, food basket estimates for the theoretical maximum daily intake were performed. Data showed that total residues intake from tissues and milk depleted below the ADI at day 1 post treatment already. Total residue intake based on residues in cattle tissues at day 1 post dose (374.1µg) and milk at 24h post dose (91.5µg, peak value) was found to be 64.7% of the ADI. The combination sheep tissues (689.4 µg) and milk (81.0µg) was found to slightly exceed the ADI (107%). This, however, was considered acceptable because of the extensive metabolism of morantel, where most of the total radioactive residues were shown to be composed of inactive, highly polar metabolites due to ring cleavages and gluthation conjugation. Furthermore, a portion of these residues was the result of incorporation into endogenous molecules and was thus no longer pharmacological-toxicologically active.

Based on data for a multiple dose regimen in cattle (bolus treatment or simulated bolus treatment), the potential intake of total residues from tissues of animals slaughtered on different occasions during treatment (between day 1 and 120) was lower than that for the single dose treatment and was estimated to be between 8 to 35% of the ADI.

22. Ruminant species such as bovine, ovine and caprines share a similar gastro-intestinal physiology. The available pharmacokinetic and residues depletion data do not indicate any significant variability between cattle and sheep, therefore, it was considered that other ruminants were unlikely to show any significant differences in these parameters. The existing MRLs for bovine and ovine species are identical and so it was considered appropriate to recommend the extension of the MRLs so that the same MRL values would apply to all ruminants, including milk.

23. According to the available data 3-(3-3-methyl-2-thienyl) acrylic acid obtained after acid digestion did not seem a suitable marker residue as this compound results for the thiophene ring, which is extensively metabolised. In one study carried out in milk, it was shown that its concentrations were about ten-fold lower than those corresponding to the drug-related metabolites obtained after alkaline hydrolysis, the N-methyl-1,3-propanediamine compound. Therefore, N-methyl-1,3-propanediamine was retained as the marker residue.

24. A gas liquid chromatography method for monitoring residues of morantel: N-methyl-1,3-propanediamine for edible tissues and milk of cattle and sheep was available. The determination of morantel marker residue is accomplished by liquid extraction of the sample in an acidic-organic extraction solvent followed by quantification *via* gas chromatography with mass spectrometry detection. This method should be applicable to other ruminant species and therefore from this aspect extrapolation to the tissues and milk of other ruminants is possible.

Conclusions and recommendation

Having considered that:

- an ADI of 12 µg/kg bw (i.e. 720 µg/person) was established,
- morantel is quickly and extensively metabolised, detoxified and excreted ,
- no significant differences in pharmacokinetics or residue depletion were observed in bovine and ovine species,
- MRLs were previously established in bovine and ovine species; these MRLs are identical,
- an analytical method for the monitoring of residues in tissues and milk of all ruminants was available;

the Committee for Medicinal Products for Veterinary Use recommends the modification of the current entry for morantel 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
Morantel	Sum of residues which may be hydrolysed to N-methyl-1,3-propanediamine and expressed as morantel equivalents	All ruminants	100 µg/kg 100 µg/kg 800 µg/kg 200 µg/kg 50 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRLs the total theoretical intake of residues from tissues and milk will represent about 92% of the ADI of 720 µg.