COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

MEBENDAZOLE

SUMMARY REPORT (1)

1. Mebendazole is a benzimidazole anthelmintic which is used in both human and veterinary medicine. In veterinary medicine, it is administered orally to horses, at a target dose of 8.8 mg/kg bw and to sheep and goats at a target dose of 15 mg/kg bw. Mebendazole has also been used in game birds, pigs, deer, poultry and cattle, including lactating animals and laying birds, but these uses were not supported with regard to the establishment of MRLs.

    Mebendazole is authorised in a range of mono-preparations including premixes for medicated feed, pastes, tablets, liquids, granules, drenches and suspensions for oral administration. Mebendazole is also used in combination products additionally containing either metrifonate, closantel or minerals (selenium, cobalt).

2. The pharmacokinetics of mebendazole was studied in rats, mice, dogs, humans and several target species. In rats given oral doses in the range of 0.06 to 10 mg/kg bw ^{14}C-mebendazole, most of the radioactivity was recovered from the organs of the gastrointestinal tract and consisted mostly of unmetabolised mebendazole. Less than 1% of the administered radioactivity was detected in blood. Excretion was predominantly via the faeces, with 70 to 90% of the faecal radioactivity consisting of unmetabolised mebendazole. In rat liver, 1 hour after administration, 15% of the radioactivity consisted of unmetabolised mebendazole. Four hours after administration, the percentage of mebendazole had declined to 1%. Absorption in humans was increased when the same dose was given with a meal. Plasma concentrations, 2 to 4 hours later, were in the range of 27 to 42 µg/l. Significantly higher concentrations were found in a patient with cholestasis. In humans, around 9% of an oral dose of ^{14}C-mebendazole was excreted in urine and the remainder in faeces; the dose level was not stated in this study. Following intravenous and oral administration of 1.7 µg of ^{3}H-mebendazole to an adult human volunteer, the oral bioavailability was estimated to be 17%. In a further 5 volunteers, the oral bioavailability was 22%. At higher, therapeutic dose levels, oral bioavailability was only around 2%.

3. In rats, dogs and humans, the major metabolite in urine was identified as 2-amino-1*H*-benzimidazol-5-yl)phenylmethanone, formed by carbamate hydrolysis of mebendazole. The major metabolite found in rat bile was methyl[5-(1-hydroxy-1-phenyl)methyl-1*H*-benzimidazol-2-yl] carbamate, formed by ketoreduction of mebendazole. In *in vitro* studies using preparations derived from pig, rat and dog liver, the major metabolite was methyl[5-(1-hydroxy-1-phenyl)methyl-1*H*-benzimidazol-2-yl] carbamate which accounted for 50%, 58% and 93% of the total radioactivity in the extracts. The metabolite (2-amino-1*H*-benzimidazol-5-yl)phenylmethanone was present in pig liver extracts. Another *in vitro* study was carried out in hepatocytes and liver subcellular fractions of rat, dog, goat, sheep, horse, cattle and human. Two major metabolic pathways were identified: ketoreduction, leading to methyl[5-(1-hydroxy-1-phenyl)methyl-1*H*-benzimidazol-2-yl] carbamate, the major metabolite in rat, dog, goat, sheep, horse and cattle subcellular liver fractions, and carbamate hydrolysis to (2-amino-1*H*-benzimidazol-5-yl)phenylmethanone, an important metabolite following incubations with horse and human hepatocytes.

4.   The metabolism of mebendazole was similar to that of its fluoro-derivative, flubendazole and involved ketoreduction, carbamate hydrolysis and conjugation as the main metabolic pathways.

5.   The oral LD_{50} values of mebendazole were 714 and 1434 mg/kg bw in female and male Wistar rats, respectively. The acute oral LD_{50} values in Swiss Albino mice, guinea pigs and New Zealand White rabbits were all greater than 1280 mg/kg bw. Diarrhoea and emesis were the most frequent signs of toxicity in the oral studies. After intramuscular injection, there were no deaths in Wistar rats given doses up to 350 mg/kg bw nor in Beagles given up to 2000 mg/kg bw; the overt signs of toxicity in these studies included head-twitching, scratching, exophthalmos and piloerection.

6.   In a 13-week study, groups of Wistar rats were fed diets equivalent to 0, 7.8, 32.1 or 127.3 mg/kg bw/day in males and 8.4, 33.7 or 151.6 mg/kg bw/day in females, respectively. At the top dose level, mortality was increased, body weight gain was reduced and changes indicative of anaemia were observed in haematology values. Serum alkaline phosphatase concentrations were significantly increased at the top dose. At termination, mean gonad weights were significantly reduced in both the mid- and top-dose group. Histopathological changes found in the testes of the top dose group included tubular degeneration and desquamation and the absence of spermatogenesis. Relative liver weights were significantly increased in the mid- and top-dose groups. Histopathological examination revealed centrilobular swelling, hepatocellular vacuolation and bile duct proliferation. The NOELs were 7.8 and 8.4 mg/kg bw/day in males and females, respectively.

Beagle dogs were given oral doses of 0, 2.5 or 10 mg/kg bw/day of mebendazole, in gelatin capsules, 6 days per week, for 13 weeks. Another group of dogs was given 0.63 mg/kg bw/day from weeks 1 to 7 followed by 40 mg/kg bw/day from weeks 8 to 13. In the group given 10 mg/kg bw/day, haemoglobin and erythrocyte counts were reduced, serum alkaline phosphatase, bilirubin, cholesterol and total protein concentrations were significantly increased and relative liver weight was significantly increased. Similar though less marked effects were observed in the group given 0.63 and then 40 mg/kg bw/day. There were no pathological findings attributable to the test substance. The NOEL was 2.5 mg/kg bw/day.

In another study, Beagle dogs received oral doses of 0, 2.5, 10 or 40 mg mebendazole/kg bw/day in gelatin capsules, 6 days per week, for 24 months. One female dog died during the first week; the death was attributed to enteritis and did not seem to be substance-related. There were no overt signs of toxicity and no substance-related effects on body weight, electrocardiogram, heart rate or blood pressure. There were considerable fluctuations in haematology and clinical chemistry values but no consistent dose-related trends. Liver weights were significantly increased in the 10 mg/kg bw group but not at 40 mg/kg bw. There were no pathological findings attributable to treatment. Drug blood concentrations were not monitored in these studies so there is no explanation for the inconsistent results.

7.   In a fertility study, groups of rats were fed diets corresponding to 0, 5 10, 20 or 40 mg/kg bw/day of mebendazole. Treated males were mated with untreated females and vice-versa. Treatment of the females continued up to day 22 of gestation when the uterine contents were examined. There were no substance-related effects on fertility and no evidence of embryolethality.

8.   In a 3-generation study in rats, the F0 and F1 dams were fed diets corresponding to 0, 2.5 or 10 mg/kg bw/day of mebendazole from day 6 to 15 of gestation. There was no evidence of maternal toxicity, foetotoxicity or teratogenicity. However the study was inadequate as a multigeneration study because of the restricted treatment regimen.

9.   In a peri/post-natal study, pregnant female rats were fed diets corresponding to 0, 5, 10, 20 or 40 mg/kg bw/day of mebendazole from day 16 of gestation up to the end of lactation. The dose of 40 mg/kg bw was maternally toxic causing reduced body weight gain. The NOEL for maternal toxicity was 20 mg/kg bw. The dose of 20 mg/kg bw was foetotoxic causing reduced litter size, an increased incidence of stillbirths and reduced pup weights. The NOEL based on foetotoxicity was 10 mg/kg bw/day.

10. Mated Wistar rats were given mebendazole in the feed at doses of 0, 2.5, 10, 40 or 160 mg/kg bw/day from day 6 to 15 of gestation. Other groups were given 0.63, 2.5, 5, 10 or 40 mg/kg bw by gavage on days 7, 8, 9 or 10 of gestation. When mebendazole was given in the feed, 10 mg/kg bw/day was a NOEL for foetotoxicity and teratogenicity. However, there was clear evidence of teratogenicity, when 10 mg/kg bw was given by gavage with 5 and 17 malformed foetuses following treatment on days 9 and 10, respectively. The malformations included exencephaly, tail malformations, coelosomy, malformed ribs and legs, and scoliosis. Teratogenicity was most marked after treatment on day 10 of gestation. The numbers of malformations in the 2.5 and 5 mg/kg bw groups were not significantly different from controls.

In a second rat teratology study, the dams received 0, 2.5, 10 or 40 mg/kg bw/day from day 6 to 15 of gestation. Maternal toxicity was observed in all treated groups. There were no live foetuses in the 40 mg/kg bw group. Litter size and pup weight were reduced at 10 mg/kg bw. Thirty one out of 133 foetuses in the 10 mg/kg bw group were malformed. The malformations included coelosomy, fused ribs, scoliosis and short or no tail. The NOEL was 2.5 mg/kg bw/day.

Pregnant mice were given daily oral doses of 0, 2.5, 5, 10 or 40 mg mebendazole/kg bw/day from day 6 to 15 of gestation. The dose of 40 mg/kg bw caused severe maternal toxicity with the deaths of 8 dams. Maternal body weight gain was reduced at 5 mg/kg bw and above. All foetuses in the 40 mg/kg bw group were resorbed and the incidence of resorptions was increased at 10 mg/kg bw. Foetal body weights were reduced at 5 mg/kg bw and above. The incidence of malformations was significantly increased in the 10 mg/kg bw group. The malformations included exencephaly, hydrocephalus, dilated ventricles, cleft palate, heart, liver, kidney and skeletal malformations. The NOEL was 2.5 mg/kg bw/day based on maternal toxicity and foetotoxicity.

Although there was no evidence of teratogenicity or foetotoxicity in hamsters given mebendazole from day 6 to 10 of gestation, the study was inadequate because the dams were not treated for the full period of organogenesis.

Inseminated rabbits were given daily oral doses of 0, 10 or 40 mg mebendazole/kg bw/day from day 6 to 18 of gestation. One dam given 40 mg/kg bw died and dams given 10 and 40 mg/kg bw gained less weight than the controls. Litter size appeared to be reduced at 40 mg/kg bw but there was no statistical analysis. There was no evidence of teratogenicity. Details of skeletal variations were not reported so no conclusion can be drawn regarding a NOEL for foetotoxicity.

11. Reproductive toxicity of mebendazole was studied in several animal species i.e dogs, cats, sheep, pigs and horses. There was no clear evidence of teratogenicity. However the studies were of limited value and suffered from a number of designs faults such as small group sizes, lack of controls and inadequate record keeping.

12. According to a brief published summary, a metabolite of mebendazole (methyl[5-(1-hydroxy-1-phenyl)methyl-1*H*-benzimidazol-2-yl] carbamate) which was found in rat bile and urine, sheep liver and *in vitro* studies using preparations of pig and dog liver was teratogenic causing external malformations in 5.4% and 100% of foetuses following oral administration of 7.4 and 9.9 mg/kg bw, respectively, to pregnant female rats on day 8 to 15 of gestation.

13. Although weak mutagenic activity was detected in the presence of metabolic activation in 1 *in vitro* assay for gene mutation in *Salmonella typhimurium* strains TA 100 and TA 98, clear negative results were obtained when the study was repeated under the same conditions. In addition, clear negative results were obtained in several other studies using these strains and also TA 1530, TA 1535, TA 1538, TA 1537 and TA 97. According to a brief published report, mebendazole was clastogenic in cultured cells and bone marrow from orally treated rats; the dose levels were not stated and no further details about the experiment were provided.

According to another published report, mebendazole induced a dose-related mitotic arrest in cultured human peripheral blood lymphocytes over the concentration range 1 to 100 µg/ml. In a published *in vitro* assay, mebendazole induced significant dose-related increases in binucleated lymphocytes with micronuclei. Negative results were obtained in an *in vivo* micronucleus test in which mice were given single oral doses of 2.5, 10 or 40 mg mebendazole/kg bw, however the top dose used in this study was too low. Negative results were reported in four different dominant lethal assays in mice and in a spermatocyte test and in an F1 translocation assay in mice using doses up to 640 mg/kg bw. The 2 latter tests are not recognised validated assays. These data suggested that mebendazole was not a germ cell mutagen.

Studies were conducted to investigate the aneugenic potential of mebendazole. Two *in vivo* bone marrow micronucleus studies were conducted in mice. In the first study, groups of 8 males received single oral doses of 0, 500, 1000 or 2000 mg mebendazole/kg bw, the animals were killed 24 hours after treatment and ratios of polychromatic (PCE) to normochromatic (NCE) erythrocytes and incidence of micronuclei were determined from femoral bone marrow smears. Fluorescence *in situ* hybridisation, also known as FISH test, was performed on additional slides from two animals in the 500 mg/kg bw group, using a probe specific for all mouse centromeres. Mebendazole treated groups showed lower PCE:NCE ratios than controls and statistically significantly increased frequencies of micronucleated PCEs (control group, 0.38; 500 mg/kg bw group, 22.5; 1000 mg/kg bw group, 27.63 and 2000 mg/kg bw group, 18.0). FISH analysis indicated that almost all mebendazole-induced micronuclei carried a centromere, indicating they were formed by aneuploidy from whole chromsomes rather than acentric fragments. In the second study, groups of 7 males were given single oral doses of 2.5 to 320 mg/kg bw, in order to establish a threshold dose for induction of micronuclei. No statistically significant increases in micronucleated PCEs were observed at 2.5 and 10 mg/kg bw (0.64 and 0.43, respectively), compared to controls (0.29). Statistically significant increases were observed at doses of 10 to 320 mg/kg bw (1.36 to 10.86).

Two *in vitro* studies were conducted to investigate induction of micronuclei by mebendazole in cultured human female peripheral lymphocytes. In the first study, incidence of micronucleated binucleate cells was determined in cultures of cells treated with 178.5, 422.5 or 650 ng mebendazole/ml. The test material was added 24 hours after the start of culture, cytochalasin B added after a further 20 hours, and cultures harvested, stained and scored at 72 hours. The frequency of micronuclei was not significantly different from controls (1.75%) at 178.5 ng/ml (2.45%), but significantly increased at 422.5 (11.6%) and 650 ng/ml (17.3%). FISH analysis of cells treated with 422.5 ng/ml with a pan-centromeric probe indicated that the majority of mebendazole-induced micronuclei were from whole chromosomes rather than acentric fragments. In a third phase to this study, FISH analysis with chromosome specific (17 and X) probes indicated the mebendazole caused chromosome non-disjunction, with clear increases in incidence over controls at 178.5 ng/ml but not at 2.403 or 20.71 ng/ml. In the second *in vitro* study, dose-response data were generated in order to determine a no effect level. Chromosomes 17 and X were examined by FISH analysis with specific probes after treatment with mebendazole at 17 concentrations ranging from 10 to 250 ng/ml. Non-disjunction again proved to be the most sensitive indicator of aneuploidy, and no-effect concentrations of 85 and 115 ng/ml were determined from chromosomes 17 and X, respectively.

These studies indicate that although mebendazole is not a direct-acting mutagen or clastogenic, it is aneugenic in mammalian somatic cells. It was not possible to identify an NOEL for aneugenicity from the *in vivo* study, but a threshold concentration for aneugenicity was identified from the results of the *in vitro* FISH studies with a no-effect concentration of 85 ng/ml.

14. A carcinogenicity study was carried out in groups of 50 male and 50 female Wistar rats were fed diets intended to provided 0, 10, 20 or 40 mg mebendazole/kg bw/day for 23 months. A carcinogenicity study was also carried out in which groups of 50 male and 50 female mice were fed diets intended to provide 0, 10, 20 or 40 mg mebendazole/kg bw/day for 22 months. There was no evidence of carcinogenicity in either study, however both studies were considered inadequate due to the poor survival rate and inadequate histopathology.

15. Mebendazole is used in human medicine for the treatment of intestinal nematodes and hydatidosis. The usual oral dose is 100 mg as a single dose, which may be repeated 2 to 3 weeks later. For some tropical diseases, 100 mg may be given twice daily for 3 days. Alternatively a single dose of 500 to 600 mg may be given. The adverse effects include abdominal pain and diarrhoea. Hypersensitivity reactions are uncommon. High doses for prolonged periods can cause liver damage and bone marrow depression. Mebendazole is contraindicated in women who may be pregnant because of the risk of teratogenicity.

16. An ADI of 0.0125 mg/kg bw was established for mebendazole, based on the NOEL of 2.5 mg/kg bw/day, which was established in the 13-week repeated-dose toxicity study in dogs, and in the studies on developmental toxicity in rats and mice, and a using a safety factor of 200. The safety factor was considered justified because the dogs were treated only 6 days per week. It was noted that mebendazole caused teratogenicity after administration in the diet at 40 mg/kg bw/day and after oral administration at 10 mg/kg bw/day and it was considered that this ADI would offer a satisfactory margin of safety with respect to the teratogenic effects of the substance. It was also noted that no teratogenic effects were noted in rabbits after oral doses up to 40 mg/kg bw. In a study in which humans were given an oral dose of 25 mg mebendazole/kg bw, plasma concentrations of 27 to 42 ng/ml were reported after 2 to 4 hours. These concentrations were 2 to 3 times lower than the *in vitro* NOEL for aneugenic effects and 3 to 4 times lower than the threshold value at a dose 2000 times higher that the proposed ADI. It can therefore be concluded that the aneugenic effects of mebendazole are sufficiently covered by the toxicological ADI.

17. A horse was given a single oral dose of 4 g in the diet and a second horse was given the same dose divided over 10 consecutive days. The overall dose corresponded to 9.4 to 11.4 mg mebendazole/kg bw/day. Both horses were killed 5 days after the end of treatment. Residues of mebendazole in liver and kidney samples, determined by HPLC with UV detection, were below the limit of detection (less than 20 µg/kg).

    Another residues depletion study was carried out in 3 horses which were killed 1, 3 or 5 days after a single oral dose of 20 mg mebendazole/kg bw. Residues of mebendazole in tissues were determined using thin layer chromatography (TLC) with a scanning densitometer. Residues in muscle were below the limit of detection (less than 100 µg/kg). Residues in kidney were 360, 360 and 410 µg/kg at 1, 3 and 5 days after treatment, respectively. At the same time points, residues in liver were 180, less than 80 and 340 µg/kg respectively. Residues in fat were not determined.

    In a GLP-compliant study, horses (females and geldings) were given a single target oral dose of 8.8 mg/kg bw mebendazole. Two horses were killed 1 day after treatment and a further 8 horses were killed 28 days after treatment. The residues of mebendazole and of 2 metabolites in tissues were simultaneously determined by the proposed routine analytical method based on HPLC with MS-MS detection. The limit of quantification for each analyte in all tissues was stated to be 10 µg/kg. One day after treatment, the mean residues of mebendazole in liver, muscle, kidney and fat, were 728, 29, 16 and 57 µg/kg, respectively, the mean residues of the metabolite methyl(5-(1-hydroxy, 1-phenyl)methyl-1*H*-benzimidazol-2-yl)carbamate were 293, 84, 85 and 60 µg/kg and the mean residues of the metabolite 2-amino-1*H*-benzimidazol-5-yl)phenylmethanone were 5047, 497, 5851 and 156 µg/kg, respectively. Twenty-eight days after treatment, residues of mebendazole and methyl(5-(1-hydroxy, 1-phenyl)methyl-1*H*-benzimidazol-2-yl)carbamate were below the limit of quantification in all tissues. Twenty-eight days after treatment, quantifiable residues of (2-amino-1*H*-benzimidazol-5-yl)phenylmethanone were found in liver (mean value 182 µg/kg) and kidney (range from below 10 µg/kg up to 23 µg/kg), but not in muscle or fat.

18. Sheep were given a single oral dose of 10 mg/kg bw ¹⁴C-mebendazole and killed (1 animal per time point) 48 hours or 72 hours after treatment. Total residues declined from 5310 to 1960 µg equivalents/kg in liver, from 1300 to 650 µg equivalents/kg in kidney and from 170 to 60 µg equivalents/kg in muscle. Total residues in fat were 130 µg equivalents/kg at 48 hours, but were undetectable at 72 hours. The fraction of unmetabolised mebendazole with respect to total radioactivity was determined using the inverse isotope dilution technique; it was estimated that less than 5% of the radioactivity in liver and kidneys was mebendazole.

In another study, sheep were given a single oral dose of 15 mg/kg bw of mebendazole and killed (3 animals per time point) at intervals up to 21 days after treatment. Residues of mebendazole in tissues were determined by HPLC with UV detection; the limit of quantification was 10 µg/kg. Mean residues of mebendazole in liver declined from 687 µg/kg at 1 day after treatment to 16 µg/kg, 3 days after treatment, and were undetectable at subsequent time points. Mean residues in kidney were 76 µg/kg at 1 day after treatment, and were undetectable at later time points. Residues in all muscle samples were below the limit of quantification. Mean residues in fat were 54 µg/kg at 1 day after treatment. Low residues of mebendazole (12 to 16 µg/kg) were found in some subsequent fat samples but residues in most subsequent fat samples were below the limit of quantification.

In another study, 2 days after an oral dose of 250 mg ^{14}C-mebendazole, 54% of the total residues in sheep liver were unextractable, 33% consisted of polar compounds and conjugates and 13% was extractable into acetonitrile and consisted of a mixture of mebendazole and free metabolites. Fifteen days after treatment, 39% of the residues were unextractable, 59% consisted of polar compounds and metabolites and 2.3% consisted of mebendazole and free metabolites. The percentage of unmetabolised mebendazole in sheep liver declined from 5.8% of the total residues, 2 days after treatment to 0.48%, 15 days after treatment. At the same time points, the metabolite methyl[5-(1-hydroxy-1-phenyl)methyl-1*H*-benzimidazol-2-yl] carbamate accounted for 3.7% and 0.12% of the residues in liver.

In a GLP-compliant study, male and female sheep were given a single oral dose of 15 mg/kg bw ^{14}C-mebendazole. The substance was formulated as the commercial product. Samples of blood and excreta were collected and the sheep were killed (4 per time point) at 1, 3, 7 or 14 days after treatment. A mean plasma C_{max} value of approximately 2.8 µg equivalents/g was attained 12 hours after treatment. Approximately 59% and 30% of the administered dose was recovered from faeces and urine respectively, over the 168-hour period post-treatment. Mean total residues in liver, muscle, kidney and fat were 14534, 1373, 7542 and 954 µg equivalents/kg, 1 day after treatment and depleted to 8069, 36, 694 and 59 µg equivalents/kg at 3 days after treatment and 3160, 11, 123 and 13 µg equivalents/kg at 7 days after treatment. In liver, muscle, kidney and fat the percentage of bound residue was 26%, 5%, 8% and 2%, 1 day after treatment, and increased to 68%, 43%, 37% and 25% at 3 days after treatment. At 7 and 14 days after treatment, the percentage of bound residues was 87% in liver and 74 to 78% in kidney but the percentage could not be determined in muscle and fat at these time points due to the low residues present. The composition of the residues was investigated using radio-HPLC and TLC profiling. One day after treatment, HPLC indicated that mebendazole represented 8%, 3%, 20% and 30% of the total residues in liver, muscle, kidney and fat respectively. At this time point, the metabolite methyl(5-(1-hydroxy,1-phenyl)methyl-1*H*-benzimidazol-2-yl)carbamate accounted for 47%, 90%, 14% and 67% of the total residues in these tissues. Three days after treatment, residues of mebendazole and the metabolite methyl(5-(1-hydroxy, 1-phenyl)methyl-1*H*-benzimidazol-2-yl)carbamate accounted for 2% and 3.5% of the total residues in liver but neither substance was detectable in liver samples taken 7 or 14 days after treatment, nor in kidney samples taken 3 days after treatment. In kidney, the metabolite M3, formed by ketoreduction accounted for 15% and 16% of the total residues 1 and 3 days after treatment; this metabolite represented 5% and 3% of the residues in liver at these time points and was undetectable in muscle and fat. The results obtained using TLC were very similar to those obtained using HPLC.

In a GLP-compliant study, male and female sheep were given a single oral dose of a commercial product at a target dose of 20 mg/kg bw mebendazole. Groups of 2 male and 2 female sheep were slaughtered 1, 7, 14, 21 and 28 days after treatment. The residues of mebendazole and of 2 metabolites in tissues were simultaneously determined by the proposed routine analytical method based on HPLC with MS-MS detection. The limit of quantification for each analyte in all tissues was stated to be 10 µg/kg. One day after treatment, in liver, muscle, kidney and fat, the mean residues of mebendazole were 1016, 21, 1460 and 343 µg/kg and the mean residues of the metabolite methyl(5-(1-hydroxy, 1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate were 7582, 1783, 1531 and 758 µg/kg. One day after treatment, residues of the metabolite (2-amino-1H-benzimidazol-5-yl)phenylmethanone were 18 and 147 µg/kg in liver and kidney (mean values), were below the limit of quantification in fat, and ranged from below the limit of quantification to 16 µg/kg in muscle. At later time points, residues of all 3 analytes were below the limit of quantification in most samples of muscle and fat. In kidney samples taken 7 days after treatment, quantifiable residues of mebendazole and methyl(5-(1-hydroxy, 1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate were found in only 1 (out of 4) samples (27 and 38 µg/kg, respectively). Seven days after treatment, the mean residues of (2-amino-1H-benzimidazol-5-yl)phenylmethanone in kidney were 44 µg/kg and this metabolite was undetectable in kidney samples at later time points. Seven days after treatment, the mean residues of mebendazole, methyl(5-(1-hydroxy, 1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate and (2-amino-1H-benzimidazol-5-yl)phenylmethanone in liver were 56, 272 and 27 µg/kg respectively. Twenty-eight days after treatment, mean residues of methyl(5-(1-hydroxy, 1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate in liver were 115 µg/kg but residues of mebendazole and the other metabolite were below the limit of quantification.

19. Male and female goats were given a single oral dose of 20 mg/kg bw mebendazole. Two goats were killed 1 day after treatment and a further 8 goats were killed 28 days after treatment. The residues of mebendazole and of 2 metabolites in tissues were simultaneously determined by the proposed routine analytical method based on HPLC with MS-MS detection. The limit of quantification for each analyte in all tissues was stated to be 10 µg/kg. One day after treatment, in liver, muscle, kidney and fat, the mean residues of mebendazole were 1020, 27, 656 and 157 µg/kg, the mean residues of the metabolite methyl(5-(1-hydroxy,1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate were 7502, 2773, 2178 and 500 µg/kg and the mean residues of the metabolite
(2-amino-1H-benzimidazol-5-yl)phenylmethanone were 121, 50, 550 and below the limit of quantification, respectively. Twenty-eight days after treatment, residues of mebendazole and the 2 metabolites were undetectable in all samples of kidney, muscle and fat. 28 days after treatment, residues of methyl(5-(1-hydroxy,1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate in liver ranged from below the limit of quantification to 308 µg/kg and residues of (2-amino-1H-benzimidazol-5-yl)phenylmethanone in liver ranged from below the limit of quantification to 19 µg/kg.

20. In the marker residue depletion study in horses, the metabolite 2-amino-1H-benzimidazol-5-yl)phenylmethanone, formed by carbamate hydrolysis, was a major component of the residues in horse tissues. In the marker residue depletion studies in sheep and goats, the metabolite methyl(5-(1-hydroxy,1-phenyl)methyl-1H-benzimidazol-2-yl)carbamate, formed by ketoreduction, was the major component of the residues in tissues. Ideally, for the sake of consistency the same marker residue should be retained for all species. It was therefore concluded that the marker residue should be the sum of mebendazole + methyl (5-(1-hydroxy, 1-phenyl) methyl-1H-benzimidazol-2-yl) carbamate + (2-amino-1H-benzimidazol-5-yl) phenyl methanone. In the radiolabelled study in sheep, this marker residue represented 93%, 97% and 38% of the total residues in ovine muscle, fat and kidney, 1 day after treatment and represented 55% and 5.5% of the total residues in ovine liver, one and 3 days after treatment respectively. The studies showed that in all species residues were most persistent in the liver. In muscle, fat and kidney, the residues were rapidly depleted; MRLs for these tissues could be set at a value of twice the limit of quantification, leaving the remainder of the ADI to be allocated to liver.

21. The proposed routine analytical method was based on HPLC with MS-MS detection. The method was capable of simultaneously determining residues of mebendazole and the metabolites: methyl(5-(1-hydroxy, 1-phenyl)methyl-1*H*-benzimidazol-2-yl)carbamate and (2-amino-1*H*-benzimidazol-5-yl)phenylmethanone in edible tissues. The limits of quantification for each analyte appeared to be 10 µg/kg for the edible tissues of horses, goats and sheep. Although values for accuracy and precision were just about acceptable for mebendazole, the values for the metabolites were not in accordance with the recommendations contained in Volume VI of the Rules Governing Medicinal in the European Community.

Conclusion and recommendation

Having considered that:

- an ADI of 0.0125 mg/kg bw (i.e. 0.75 mg/person) was established,
- the sum of mebendazole, methyl(5-(1-hydroxy, 1-phenyl)methyl-1*H*-benzimidazol-2-yl)carbamate and (2-amino-1*H*-benzimidazol-5-yl)phenylmethanone was identified as the marker residue,
- the marker residue represented 93%, 97% and 38% of the total residues in ovine muscle, fat and kidney, 1 day after treatment, and 5.5% of the total residues in ovine liver, 3 days after treatment,
- MRLs for muscle, fat and kidney could be set at twice the limit of quantification,
- a routine analytical method is available but not fully validated;

the Committee for Veterinary Medicinal Products recommends the inclusion of mebendazole into 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
Mebendazole	Sum of mebendazole, methyl(5-(1-hydroxy, 1-phenyl)methyl-1*H*-benzimidazol-2-yl) carbamate and (2-amino-1*H*-benzimidazol-5-yl) phenylmethanone, expressed as mebendazole equivalents	Ovine, caprine, equidae	60 µg/kg 60 µg/kg 400 µg/kg 60 µg/kg	Muscle Fat Liver Kidney	Not for use in animals from which milk is produced for human consumption. Provisional MRLs expire on 1.1.2002

Based on these MRL values, the daily intake amounts to 757 µg/day. Although this slightly exceeds the ADI, this was considered to be acceptable because of the low oral bioavailability of mebendazole in humans.

LIST OF QUESTIONS

1. The Applicant should fully validate the proposed routine analytical method for the identified marker residue to obtain values for accuracy and precision, for all the target species, in accordance with the recommendations of Volume VI of the Rules Governing Medicinal Products in the European Community and also taking into account the agreed CVMP Note for Guidance on the Establishment of Maximum Residue Limits for Minor Animal Species (Doc. EMEA/CVMP/153a/97-FINAL).