European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

THIABENDAZOLE
(Extrapolation to goats)

SUMMARY REPORT (3)

1. Thiabendazole [2-(1,3-thiazol-4-yl)benzimidazole] is a benzimidazole anthelmintic used in the treatment and control of gastrointestinal roundworms and lungworms in ruminants which is currently 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
Thiabendazole	The sum of thiabendazole and 5-hydroxy-thiabendazole	Bovine	100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	

2. In reviewing the availability of endo- and ectoparasiticides for sheep and goats, thiabendazole was considered for extrapolation from bovine species to goats. The considerations and criteria leading to the identification of thiabendazole are described in the Position Paper Regarding Availability of Veterinary Medicines – Extrapolation of MRLs (EMEA/CVMP/457/03-FINAL).

3. The scientific justification for this extrapolation was assessed in accordance with the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL).

4. In setting the ADI in the original assessment of thiabendazole, the data summarised in the paragraphs below the were considered.

5. Thiabendazole and other benzimidazole anthelmintics act by binding strongly to tubulin in the absorptive cells in the gut of parasitic worms. This interferes with the uptake of nutrients and the worms effectively starve to death. The host is less affected as the binding to mammalian tubulin is less strong and is reversible. Another pharmacological study showed that single oral doses of 25 to 50 mg/kg bw reduced oedema in the paws of rats and decreased fever. A pharmacological NOEL was not identified.

6. Investigations in mice, rats and dogs using ^{14}C-labelled thiabendazole indicated that oral doses were rapidly absorbed from the gut and were distributed throughout the body (including the brain). Only 0.01% of the ^{14}C-thiabendazole given to rats was recovered as ^{14}C-carbon dioxide. Thiabendazole readily crossed the placental barrier to expose the foetuses.

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Peak plasma concentrations in pregnant mice were achieved within 30 minutes of gavage administration using an oily vehicle and after 6 hours when aqueous gum arabic was used in rats and the peak level was achieved at 2 to 3 hours after treatment; in dogs it was achieved within 2 hours. In humans, peak plasma concentrations were observed 1 hour after administration of thiabendazole, then they rapidly decreased, reaching zero after 24 to 48 hours. After intake of oral doses of 1000 mg/person of ^{14}C-thiabendazole by men, plasma peak plasma concentrations of 1 to 6 µg/ml thiabendazole, 5 to 10 µg/ml 5-hydroxythiabendazole and 9 to 15 µg/ml total benzimidazoles were achieved. In mice and rats, the radioactivity was recovered more in the urine than in the faeces, whereas in dogs faecal recovery was slightly greater than urinary. In humans, urinary excretion predominated (81 to 91% of dose recovered in urine; 2 to 7% in faeces).

7. In mice, rats and humans, the main pathway of metabolism of thiabendazole is an initial hydroxylation to form 5-hydroxythiabendazole, followed by conjugation to 5-hydroxy-thiabendazole glucuronide and 5-hydroxythiabendazole sulphate. In rats, 4-hydroxythiabendazole and 2-acetylbenzimidazole have been identified as minor metabolites or degradation products in urine.

8. Thiabendazole is of low acute toxicity by the oral route. Oral LD_{50} values were in the range 2400 to 3810 mg/kg bw in mice, 3300 to 8600 mg/kg bw in rats, and 3850 mg/kg bw in rabbits. By the intraperitoneal route, LD_{50} values were 430 mg/kg bw in mice and 1850 mg/kg bw in rats. Intravenous LD_{50} values were 130 mg/kg bw in mice and 150 mg/kg bw in rats. Signs of acute toxicity seen at high doses included bodyweight loss, inanition, ataxia, narcosis and central nervous system depression, with subsequent death from respiratory failure. In dogs, acute oral doses of 200 or 300 mg/kg bw or an intravenous dose of 25 mg/kg bw caused emesis. A single oral dose of 4000 mg/kg bw produced no effect on blood pressure, electrocardiogram or respiration in dogs or cats.

9. Mice given thiabendazole in oral gavage doses of 250 or 500 mg/kg bw/day for up to 7 days showed renal damage to the tubules and glomeruli. In pilot studies of 3 and 6 weeks duration, mice were fed diets which gave them doses of 0, 50, 150, 300, 600 and 900 mg/kg bw/day of thiabendazole with no effect on mortality or clinical appearance, but food intake and bodyweight gain were decreased at doses of 600 mg/kg bw/day or more.

   In a 13-week mouse feeding study, concentrations of 0, 8000, and 16000 mg/kg of thiabendazole were given. This gave dosages of approximately 0, 1450 and 2300 mg/kg bw/day in males and 0, 1650 and 2800 mg/kg bw/day in females. Bodyweight was significantly decreased in all groups of treated males and in the top dose females, but food intake was decreased only in top dose males. Haematology showed decreases in red blood cell parameters (rbc, pcv, MCHC, MCH) which affected all treated groups. These groups also had an increased platelet count and a mild neutrophilia with concurrent lymphopaenia. Serum transaminases (ALT and AST) were raised in all treated groups. Weights of liver, spleen and kidneys were raised in most treated groups and there was centrilobular hepatocellular necrosis, bile duct proliferation, splenic haemosiderosis, necrosis, fibrosis and atrophy of renal tubules and urothelial hyperplasia. A NOEL was not identified for this study.

10. In a 30-day study, which was not performed to current standards, rats were given oral gavage doses of 0, 100, 400, 800, 1200 or 1600 mg/kg bw/day. Hepatotoxicity and anaemia were seen at 400 mg/kg bw/day or more. A NOEL was not identified for this study as decreased bodyweight gain was seen at all doses.

    Rats given 2500 or 5000 mg/kg in their diet for 6 weeks showed anaemia and hepatomegaly.

    Rats were fed thiabendazole at dietary concentrations of 0, 500, 1000, 2000, 4000 or 8000 mg/kg for 13 weeks. At dietary levels of 4000 mg/kg or more, reduced bodyweight gain, reduced food intake, and anaemia (reduced mean corpuscular volume, haemoglobin and packed cell volume). The total white blood cell count was also decreased at 8000 mg/kg. The NOEL for this study was 2000 mg/kg (200 mg/kg bw/day).

Rats were fed thiabendazole for 14 weeks at dietary concentrations which gave dosages of 0, 9, 37, 160 and 320 mg/kg bw/day. Bodyweight gain was reduced at dosages of 37 mg/kg bw/day or more. At 160 and 320 mg/kg bw/day erythrocyte count was decreased whilst serum cholesterol was increased. At 37 mg/kg bw/day or more, liver and thyroid weights were increased and there was centrilobular hepatocellular hypertrophy, thyroid follicular cell hypertrophy and bone marrow erythroid hyperplasia. The NOEL for this study was 9 mg/kg bw/day.

In a study not performed to current standards, rats were given gavage doses of 0, 25, 100 or 400 mg thiabendazole/kg bw/day for 14 weeks. At the two highest doses, there was reduced bodyweight gain, anaemia, increased serum cholesterol, increased liver weight with centrilobular hepatocellular hypertrophy, enlarged thyroids with follicular cell hyperplasia, renal calculi, renal transitional epithelial hyperplasia, degeneration of the non-glandular stomach epithelial cells, and splenic haemosiderosis. The NOEL for this study was 25 mg/kg bw/day.

In a 6-month study, rats were given oral gavage doses of 0, 12.5, 25, 50, 100, 200 or 400 mg/kg bw/day. At 200 mg/kg bw/day or more, there was a depression of bodyweight gain. At 400 mg/kg bw/day, haematology showed a lowering of the red blood cell parameters, lymphopaenia and neutrophilia. There was also bone marrow hypoplasia and elevated serum alkaline phosphatase at this top dose. Enlarged livers and thymic haemosiderosis were seen in both sexes at doses of 100 mg/kg bw/day or more, and females given 50 mg/kg bw/day also had enlarged livers. Colloid depletion was seen in the thymuses of rats given 200 mg/kg bw/day or more. The NOEL for this study was 25 mg/kg bw/day.

11. Beagle dogs were given daily oral doses of 0, 35, 75 or 150 mg/kg bw/day of thiabendazole in gelatin tablets for 14 days. In all groups including controls there was cytoplasmic vacuolation of the gallbladder epithelium, but the incidence and severity was highest in the mid and high dose groups. At the highest dose there was also an increased relative liver weight (without corresponding histopathology) and anaemia. The NOEL for this study was 35 mg/kg bw/day.

    Beagle dogs were given daily oral doses of 0, 10, 40 or 160 mg/kg bw of thiabendazole for 53 weeks. Some haematological changes were detected in the high dose group: erythrocyte count, haemoglobin, packed cell volume were decreased, whereas the number of nucleated red blood cells, polychromasia, hyperchromia, activated partial thromboplastin time and platelet number were all increased. There were increased incidences in all treated groups of inspissated bile between villi and of cytoplasmic vacuolation of the epithelium at the tips of villi of the gall bladder. Liver weights were significantly increased in the mid and high dose groups (sexes not analysed separately), but there was no associated histopathology. Thyroid weights were slightly increased but they were not statistically significantly different to concurrent control values. Thyroid follicular cell hyperplasia was seen in some dogs in the group given 160 mg/kg bw/day, but was not seen in any other group. Increased incidences of epithelial vacuolation of the renal distal tubules were found in mid and high dose females. In both sexes at the mid and high doses, there were increased incidences of splenic erythropoiesis, splenic haemosiderin and of cytoplasmic inclusions (resembling lipofuscin) in the epithelium of the urinary bladder. Splenic haemosiderosis was also found in half of the male dogs in the low dose group. It was clear that doses of 40 mg/kg bw/day or more had adverse effects, but at 10 mg/kg bw/day the only effects seen were minor changes to the gall bladder and spleen. These effects were considered to be unrelated to any inherent toxicity of thiabendazole. None of the effects seen at the lowest dose were considered to be treatment-related and therefore the NOEL for this study was regarded as being 10 mg/kg bw/day.

    In a study (not to current standards) using only 2 animals per sex per group, beagle dogs were given oral doses of 0, 20, 100 or 200 mg/kg bw/day for 2 years. At the highest dose there was a retardation of bodyweight gain and a decreased erythrocyte count. At 100 and 200 mg/kg bw/day there was haemosiderosis in the spleen, liver, lymph nodes and bone marrow. The NOEL for this study was 20 mg/kg bw/day.

    In another 2-year dog study (3 animals per sex per group) which was not to current standards, beagle dogs were given oral thiabendazole doses of 0, 20, 50 or 125 mg/kg bw/day. Mortality was increased at the top dose level and moderate chronic inflammation was seen in the livers of most of the dogs in this group. At 50 mg/kg bw, there was a decreased bodyweight gain in males only.

Haemoglobin and packed cell volume were decreased in the mid and top dose groups at various times throughout the study. The NOEL for this study was 20 mg/kg bw/day.

In a dog study which was not to current standards, beagle dogs were given gradually increasing oral doses of thiabendazole over 127 days until doses of 0, 10, 100 and 200 mg/kg bw/day were reached. Thereafter, these doses were fed for a further 104 weeks. Increased weights (relative and absolute) of adrenals, liver and kidneys were seen at the two highest doses and haemosiderosis was apparent in some organs. At the highest dose, there was reduced bodyweight gain and anaemia. The NOEL for this study was 10 mg/kg bw/day.

12. When female mice were fed a dietary concentration of 2 mg thiabendazole/kg feed for 2 years, bodyweight gain was less than in concurrent controls. Treated mice had increased levels of glucose and protein in their urine and had a decreased erythrocyte count whilst the white blood cell count was raised (no effect on the differential white blood cell count). Serum guanosine triphosphate (GTP) was elevated. Splenomegaly was observed in most of the treated mice. There was no excess incidence of any type of tumour. Similar findings were made in another group of female mice which were treated with 2 mg thiabendazole/kg feed plus 5 mg diphenyl/kg feed for 2 years. In a long-term toxicity/carcinogenicity study in mice, thiabendazole was fed for up to 2 years at dietary concentrations of 0 (3 control groups), 220, 660 and 2000 mg/kg to males and at 0 (3 groups), 60, 2000 and 5330 mg/kg to females. Initially, the low dose females received a dietary level of 660 mg/kg but this was reduced to 60 mg/kg after 7 weeks. These levels of dietary incorporation gave dosage ranges of 0, 5.6 to 8.3, 63 to 121, and 184 to 372 mg/kg bw/day in males and 0, 5.7 to 9.9, 209-368 and 534 to 1005 in females. Mortality was increased in the mid and high dose groups of both sexes, and some of these groups of animals (and corresponding proportions of the control groups) were killed early, at weeks 81 and onwards, when the survival became 20% or less, myocardial thrombosis being the main cause of death in the high dose groups. Bodyweight gain was depressed in the mid and high dose males and in the high dose females. The liver weight was increased in the high dose group animals of both sexes, and kidney weights were lowered in the mid and high dose males and in the high dose females. A high incidence of arterial thrombosis was seen in the 2000 mg/kg mice of both sexes and in the 5330 mg/kg females. No other treatment-related lesions were seen. Tumour incidences and time of onset were not affected by the treatment with thiabendazole. The NOELs for this study were dietary concentrations of 220 and 60 mg/kg feed for males and females respectively, which corresponded to ranges of dosages of 5.6 to 8.3 and 5.7 to 9.9 mg/kg bw/day.

13. In a long-term rat feeding study which was not to current standards, thiabendazole was given for 2 years at dietary concentrations to achieve dosages of 0, 10, 40 and 160 mg/kg bw/day. Bodyweight gain was decreased at the top dose. Thyroid and pituitary weights were increased in top dose males, but no treatment-related histopathology was seen. Haematology, clinical chemistry and urinalysis were unaffected by treatment. The NOEL for this study was 40 mg/kg bw/day.

    In a long-term toxicity/carcinogenicity study, rats were fed diets containing thiabendazole at dosages of 10, 30 and 90 mg/kg bw/day for 106 weeks. Two control groups were given unmedicated feed only. The mortality in this study (40 to 2% in males; 52 to 64% in females) was higher than normally expected in 2-year rat studies, but was no greater in treated groups than in the control groups. Decreases in food intake and bodyweight gain were seen in mid and high dose males and in high dose females. Decreases in erythrocyte count, blood haemoglobin concentration and packed cell volume were seen in both sexes at the highest dose level. Relative (to bodyweight) weights of liver and thyroid were increased in high dose males and females, respectively. Histological examination revealed significantly increased incidences of centrilobular hepatocellular hypertrophy in males in the mid and high dose groups and increased incidences of renal pelvic epithelial hyperplasia in mid and high dose females and high dose males. There were increased incidences of thyroid follicular adenomas in mid and high dose males and in high dose females, although the increase was only statistically significant in the high dose males. There was also increased incidences of diffuse follicular hypertrophy and/or focal cystic follicular cell hyperplasia in the thyroids of rats in the mid and high dose groups. The incidences of other tumours were unaffected by treatment. The NOEL for this study is 10 mg/kg bw/day.

14. In a study to investigate the mechanism of the thyroid tumourigenicity seen in rats, groups of rats were given thiabendazole in their food at concentrations to achieve dosages of 0, 10, 90 and 270 mg/kg bw/day for 13 weeks. During the treatment period, serum T_3 concentrations were significantly decreased in the mid and high dose groups (although there was no statistically significant effect on serum thyroxine) and there were also large significant increases in serum thyroid stimulating hormone (TSH) in the same groups. The necropsies at the end of the treatment period showed significant dose-related increases in relative liver weights (with centrilobular hepatocellular hyperplasia) and significant dose-related increases in thyroid weight (with diffuse follicular cell hyperplasia) in mid and high dose groups. In the rats killed after a 13-week recovery period, the thyroid and liver weights in all the treated groups were similar to the control values and no hyperplasia was evident in these organs. Thyroxine clearance data showed the high dose group to have a significant 44% increase in thyroxine clearance as compared with controls (statistically significant), but the clearance in low and mid doses was not statistically different to that in controls.

    The development of thyroid adenomas was considered to be consequent to thiabendazole causing liver enlargement, which led to increased clearance of thyroid hormones. The resultant low serum thyroxine and/or T_3 led to compensatory thyroid follicular cell hyperplasia to produce more thyroid stimulating hormone. Over time, with continued thyroid stimulation, the follicular hyperplasia progressed to follicular adenomas. The NOEL for this study is 10 mg/kg bw/day.

15. A large amount of mutagenicity data are available. They indicate that thiabendazole does not produce gene mutations in bacterial tests (no mammalian cell gene mutation tests available), structural damage to chromosomes *in vitro* (metaphase analyses), nor in Syrian hamster embryo (SHE) cell transformation assays, but the results of tests in fungal and mammalian cell systems (including *in vitro* metaphase analyses, *in vitro* micronucleus/kinetochore assays, induction of c-mitoses, and yeast aneuploidy assays) provide consistent evidence of aneugenicity *in vitro*. The *in vitro* aneugenicity of thiabendazole has been shown in a series of *in vitro* experiments to be a consequence of it binding to tubulin, which results in dysfunction of spindle mechanism at cell division. All of the validated oral route *in vivo* mutagenicity assays in either somatic cells (bone marrow metaphase analyses, and bone marrow micronucleus tests) or germ cells (dominant lethal assays) were interpreted as being negative. There were some reports of aneuploidy in bone marrow cells *in vivo* following intraperitoneal administration of thiabendazole to mice and also in one bone marrow micronucleus study in mice where the route of administration was not specified and in one investigation of sister chromatid exchange in the peripheral lymphocytes of monkeys. The conduct and standard of reporting of these apparently positive studies were considered to be inadequate by contemporary standards. Since the great weight of evidence indicated that thiabendazole did not cause any *in vivo* genotoxic effects when it was administered by the oral route, it was concluded that thiabendazole residues in food do not give rise to concern with respect to mutagenic hazard to consumers.

16. A 5-generation reproduction study in mice was reported only in a brief summary. Dietary concentrations of 200, 1000 or 5000 mg thiabendazole/kg feed were given. At 1000 mg/kg or more, there was a decrease in the bodyweights of foetuses at weaning. At 5000 mg/kg, there also were reductions in the litter size and the number of offspring weaned. The NOEL for this study was 200 mg/kg feed (equivalent to 30 mg/kg bw/day).

17. In a 2-generation reproduction study, groups of rats were fed dietary concentrations of thiabendazole at dosages of 0, 10, 30 and 90 mg/kg bw/day. In the mid and high dose males and the high dose females of both the F_0 and F_1 generations, there were dose-related decreases in bodyweight gain and food consumption. The only other significant adverse effects were restricted to the top dose group: reduced bodyweight gain of F_0 dams during pregnancy and low bodyweight of F_1 female pups and of F_2 pups of both sexes prior to weaning. No adverse effects were seen on reproductive performance nor on the gross appearance of offspring. Histological examination of reproductive organs revealed no treatment related effects. The NOEL for this study is 10 mg/kg bw/day.

In a 2-generation study which was not performed to modern standards and was reported only in summary, groups of rats were fed dietary concentrations of up to 500 mg/kg of thiabendazole (equivalent to 45 mg/kg bw/day). No effects on reproductive performance (number of successful pregnancies, litter size, number of still births, bodyweights of pups at birth and at weaning) or on the incidence of abnormal young were seen at any dose. The NOEL for this study was 45 mg/kg bw/day.

A 3-generation reproduction study was performed in rats, using oral gavage dosages of 0, 20, 40, and 80 mg/kg bw/day of thiabendazole. Decreased food intake and bodyweight gain were seen in males at all dosages in the F_0 and F_1 generations and in top dose females in the F_1 and F_2 generations. No adverse effects were seen on clinical appearance of dams, histopathology of dams, reproduction performance or lactation performance. The effects seen were likely to be due to unpalatability of the diet rather than any inherent toxicity of thiabendazole. The NOEL for this study was 80 mg/kg bw/day.

18. The developmental toxicity of thiabendazole was compared in ICR mice from three different suppliers. Groups of pregnant Jcl:ICR, CRJ:CD-1(ICR) and S1c:ICR mice were given single oral doses of 0 (control), 1157 or 1389 mg/kg bw on day 9 of pregnancy. Similar types of effects were seen in all three types of mouse, but they were most severe in the Jcl:ICR mice. In comparison with controls, all treated groups had increased incidence of early resorptions, reduced number of live foetuses per litter, reduced foetal bodyweights, increased incidences of foetal abnormalities (principally short or absent tails).

    In an investigation of the effect of timing of exposure on teratogenicity, groups of Jcl:ICR mice were given single gavage doses of 2400 mg/kg bw on a single day of gestation between days 6 and 15. Mortality was increased in all treatment groups. The number of early resorptions increased and the number of late resorptions was generally low (although increased in animals treated on days 9 or 15). Foetal bodyweights were depressed and the litter size was decreased. Most of the external abnormalities of foetuses occurred in the groups dosed on day 9 (reduced limb size, short or absent tail, anal atresia), or on days 11 or 13 (cleft palate). Increased incidences of microcephaly and exencephaly were seen in groups dosed on days 6 to 8. Most of the skeletal abnormalities (fused vertebral arches, fused vertebral bodies and ribs) occurred in the groups dosed on days 7, 8 or 9. The highest incidence of abnormalities was in the day 9 group.

    Groups of Jcl:ICR mice were given single oral gavage doses of 0, 30, 60, 120, 240, 360, 480, 965, 1157, 1389, 1667, 2000 or 2400 mg/kg bw of thiabendazole on day 9 of gestation. At doses of 60 mg/kg bw or more, foetal bodyweight was decreased. The incidences of fusion of vertebral arches, vertebral bodies and of ribs, were increased at 240 mg/kg bw or more. The incidence of limb reduction deformity was increased at 480 mg/kg bw or more. Maternal bodyweight gain was reduced at 1157 mg/kg bw or more. Maternal mortality and the resorption rate were increased at 1667 mg/kg bw or more. The NOEL for this study was 30 mg/kg bw.

    Groups of Jcl:ICR mice were given daily gavage doses of 0, 700, 1300 or 2400 mg/kg bw of thiabendazole on gestation days 7 to 15. Maternal bodyweight gain was decreased in all groups in a dose-related fashion. There were reduced foetal bodyweights and increased incidences of foetal abnormalities (including open eye, cleft palate, fused vertebral bodies, fused vertebral arches) in all treated groups. A NOEL was not identified for this study.

    Jcl:ICR mice were used in a teratology study of thiabendazole. A single oral gavage dose of 0, 250, 500 or 1000 mg/kg bw was given on day 9 of gestation. Duplicate studies were performed in groups of mice given three different commercial diets, but the results obtained were similar with all the diets. Maternal bodyweight gain was decreased at all dose levels. There was a dose-related increase in skeletal deformities (including fusions of the vertebral arches, vertebral body and ribs) at all dose levels. No NOEL was identified for this study.

    When pregnant mice were given single oral doses of 0, 750 or 1500 mg/kg bw on day 16 of gestation, spontaneous abortions occurred in 3/6 mice in the high dose group and 1/6 in the low dose group and respectively 1/6 and 2/6 dams died in these groups. Higher incidences of neonatal foetal death were found in the treated groups. However, when single doses of 12.5 and 25 mg of thiabendazole (250 to 350 mg/kg bw and 500 to 600 mg/kg bw) were given to pregnant mice on days 16, 17 or 18 of gestation, no effects on pregnancy outcome were observed.

Adverse effects were seen at all doses when groups of pregnant mice were given oral doses of 700, 1300 or 2400 mg/kg bw/day of thiabendazole (controls were given vehicle alone) on days 7 to 15 of gestation. At all dose levels there was maternal toxicity (increased organ weights) and there were dose-related increases in embryonal death (mainly late) and the incidence of external abnormalities in the foetuses. The main type of abnormality seen was cleft palate. No NOEL was identified for this study.

Groups of pregnant mice were given daily gavage doses of 0, 25, 100 or 200 mg/kg bw of thiabendazole on days 6 to 15 of gestation. Maternal bodyweight gain and food consumption were significantly reduced in the mid and high dose groups. Bodyweights of both male and female foetuses and the number of implants were decreased in the mid and high dose groups. Higher incidences of cleft palate, malformed tail, and clubbed hind foot were detected in foetuses from treated groups than in controls, but as there was no dose-response relationship and the incidences were all very low (litter means of 1.3% or less) it was concluded that these observations were unrelated to treatment. Similarly, there was an increased incidence of incomplete ossification of the talus-calcaneus in all treated groups compared with controls, but as there was no dose-response relationship and as ossification at other sites was not affected, it was considered that this too was a fortuitous result. In the high dose group, 3 foetuses from the same litter had a ventricular septal defect, transposition of the great vessels, *situs inversus* and/or variation in lung lobation. The NOEL for this study was 25 mg/kg bw/day.

19. Rats were given oral gavage doses of 0, 125, 250 and 500 mg/kg bw/day of a commercial formulation containing 45% thiabendazole on days 6 to 15 of their gestation. Foetal bodyweight was significantly decreased in the low dose group but not at higher doses. There were increases in the numbers of foetuses with abnormalities in all of the treated groups but the increase was statistically significant only at the top dose. The most commonly reported abnormality was delayed ossification of the sternebrae.

    Groups of pregnant rats were given oral gavage doses of 0, 100, 200, 400 or 800 mg/kg bw/day of thiabendazole on days 6 to 17 of gestation. Maternal bodyweight gain was decreased at all treatment levels and there was increased maternal mortality at 400 mg/kg bw/day or more. There were dose-related decreases, affecting all dose groups, on litter size, litter weight, foetal bodyweight whilst there was an increase in the proportion of dams with all their embryos/foetuses resorbed. A NOEL was not identified for this study (less than 100 mg/kg bw/day).

    In order to study the effect of the timing of exposure, groups of pregnant rats were given single oral gavage doses of 200, 400 and 800 mg/kg bw of thiabendazole on different days of gestation: days 7, 9, 11 and 13. No special control groups were used, but the results were compared with the results for the two control groups used in the next study described in this document. The number of resorptions was decreased only in the rats dosed on day 7 of gestation, but there was no dose-relationship. Apart from this, there was no indication that timing of dosing had any effect.

    Thiabendazole was fed to pregnant rats at dietary concentrations of 0 (two control groups), 200, 400, and 800 mg/kg (equivalent to 0, 14, 28, and 56 mg/kg bw/day) during days 6 to 17 of gestation. Maternal bodyweight gain was decreased at all dose. The number of dams carrying live young to gestation day 20, litter weights, and foetal birth weights were decreased at all dose levels. The incidence and severity of skeletal abnormalities were increased in treated rats (not dose-related). A NOEL was not identified for this study (less than 14 mg/kg bw/day).

    There were no effects on resorption rate or foetal survival when pregnant rats were given daily oral gavage doses of 80 mg/kg bw of thiabendazole on days 8 to 15 of gestation.

    Groups of pregnant rats were fed diets containing thiabendazole at concentrations of 0, 2, 15, 50 or 100 mg/kg for days 6 to 17 of gestation. The dietary concentrations gave dosages of 0, 0.14, 1.1, 3.5 and 7 mg/kg bw/day. Foetal bodyweight was slightly but significantly reduced and there were increased numbers of resorptions at doses of 50 mg/kg or more. At these doses, there were also increased numbers of foetuses with minor skeletal anomalies and variations due to delayed ossification. The incidences of cleft palate, absence of ossa hyoideus and extra ribs were increased in the top dose group. The NOEL for this study was 15 mg/kg feed (1.1 mg/kg bw/day).

Groups of pregnant rats were given dietary concentrations of 0, 1250, 2500, 5000 and 10000 mg thiabendazole/kg feed on days 7 to 17 of gestation. This gave dosages of 0, 92, 155, 224 and 188 mg/kg bw/day. At 2500 mg/kg or more, the dams were weak, listless and displayed piloerection. Foetal bodyweight was low in the groups given 5000 mg/kg or more. There was no increase in the incidence of external abnormalities of foetuses, but skeletal abnormalities were increased at 5000 mg/kg or more. The skeletal abnormalities included variations of sternebrae and cervical arches, and hypoplasia of the skull bones. These effects were thought to be secondary to maternal toxicity.

Groups of pregnant rats were given gavage doses of 0, 50, 100, 200 or 400 mg/kg bw/day of thiabendazole on days 6 to 17 of gestation. There were severe effects on bodyweight in the top two dose groups. Maternal bodyweight gain was also depressed at 50 and 100 mg/kg bw/day. Lethargy and ptosis were observed in rats given 100 mg/kg bw/day or more. Foetal bodyweight was decreased at all dose levels, but no increased incidence of foetal abnormalities was seen at any dose.

Pregnant rats were given oral gavage doses of 0, 10, 40 or 80 mg/kg bw/day of thiabendazole on gestation days 6 to 17. Food intake, maternal bodyweight gain and foetal weight were decreased at doses of 40 mg/kg bw/day or more. Ptosis was seen in dams at 80 mg/kg bw/day. Foetal weight was decreased at 40 mg/kg bw/day or more. No increased incidences of foetal external, visceral or skeletal/cartilage abnormalities were seen at any dose. The NOEL for this study was 10 mg/kg bw/day.

20. In a rabbit teratology study which was not to current standards, oral gavage doses of 0, 100, 200, 400 and 800 mg/kg bw/day of thiabendazole were given on days 8 to 16 of gestation. At 200 mg/kg bw/day or more, maternal bodyweight gain was decreased and the resorption rate was increased. Foetal weight was decreased at 400 mg/kg bw/day or more. No increased incidence of foetal abnormalities was seen at any dose. The NOEL for this study was 100 mg/kg bw/day.

    Pregnant rabbits were given oral gavage doses of 0, 100, 200, 400, or 800 mg/kg bw/day of thiabendazole on days 6 to 18 of gestation. Food intake and maternal bodyweight gain were decreased at 200 mg/kg bw/day or more. Implantation rate was decreased at 400 mg/kg bw/day. No increased incidence of external foetal abnormalities was seen at any dose level.

    Groups of pregnant rabbits were given doses of 0, 24, 120 or 600 mg/kg bw/day of thiabendazole by oral gavage on days 6 to 8 of gestation. Maternal toxicity, as evidenced by bodyweight loss, was seen at the highest dose levels, and reduced bodyweight gain was seen at the mid dose level. There were increased numbers of resorptions at the mid and high doses, including four whole litter resorptions at 120 mg/kg bw/day and four abortions occurred at 600 mg/kg bw/day. In addition, there were 1 and 2 foetuses with domed head, hydrocephaly and enlarged fontanelles at 120 and 600 mg/kg bw/day, respectively. The NOEL for this study was 24 mg/kg bw/day.

    Groups of pregnant rabbits were given doses of 0, 50, 150 or 600 mg/kg bw/day of thiabendazole by oral gavage on days 6 to 18 of gestation. There was an adverse effect on maternal bodyweight and food intake at the 600 mg/kg bw/day dose, but there was no mortality. At the highest dose, foetal birth weight was decreased, the number of resorptions was increased, and there were significant increases in the incidences of “variations in lung lobation” and of incompletely ossified metacarpals. The NOEL for this study is 150 mg/kg bw/day.

21. The results of *in vitro* developmental studies in limb bud cell and organ cultures demonstrated that the inhibition of chondrogenesis was dependent upon the presence of the NH-group within the imidazole moiety of the thiabendazole molecule.

    Special studies have been performed to investigate the renal toxicity of thiabendazole. It was demonstrated *in vivo* that the renal toxicity was more severe in mice depleted of glutathione than in normal mice. Similar results were obtained *in vitro* using renal cortical slices, in which the addition of glutathione could completely protect against thiabendazole toxicity. The addition of inhibitors of renal microsomal cytochrome P-450-dependent monooxygenases was also protective against thiabendazole toxicity in renal slices. 5-Hydroxythiabendazole is likely to be the toxic metabolite.

22. In a non-standard skin sensitisation test, guinea-pigs were given a series of 10 intracutaneous injections (3 per week) of a 1 mg/kg suspension of thiabendazole in saline, followed 2 weeks later by an intracutaneous challenge dose of 0.5 mg/kg. The skin reaction to the challenge dose was no greater than that to the initial injections, suggesting that thiabendazole was not a potential skin sensitizer.

23. Studies of the immunological properties of thiabendazole have been performed. Repeated gavage doses of 200 mg/kg bw/day for 9 days reduced the size of lung granulomata induced by *Schistosoma mansoni* eggs but had no effect on granulomata caused by non-antigenic plastic beads. Thiabendazole suppressed cell-mediated immunity *in vivo* and enhanced the delayed hypersensitivity response to dinitrochlorobenzene. Thiabendazole, when given in combination with the T-cell-dependent antigen dintrofluorobenzene, restored the cell-mediated response in immunosuppressed mice, potentiated all stages of T-lymphocyte development in normal mice and has been successfully used to treat murine lupus.

24. In humans, no severe side effects were reported in initial clinical trials of thiabendazole. In a study of human volunteers given oral doses of either 250 mg/person/day (3 to 4 mg/kg bw/day) of thiabendazole or a placebo for 24 weeks, no adverse effects were seen on physical condition, haematology, serum clinical chemistry (including protein-bound iodine), urinalysis or electrocardiograms. Following long and extensive use of thiabendazole in human medicine at recommended dosages of up to 3000 mg/person/day (50 mg/kg bw/day in a 60 kg person), occasional reports of side effects have included the following: anorexia, hyersensitivity reactions including Stevens-Johnson syndrome, liver damage and visual disorders. Human medicinal use is contraindicated during pregnancy and breast feeding.

25. The large number of available studies indicated a range of toxicological effects of thiabendazole, including hepatotoxicity, anaemia, renal toxicity, the production of thyroid adenomas by a non-genotoxic mechanism, and reproductive toxicity. An overall NOEL of 10 mg/kg bw/day was identified, based on the weight of evidence from the most reliable studies, covering a range of toxicological end-points including effects on the liver, thyroid, and bone marrow, spleen, effects on reproductive performance, teratogenicity in mice and foetotoxicity in rats. One rat teratology study showed adverse effects on foetal development at doses below this (3.5 and 7 mg/kg bw/day), but this finding was not consistent with the results of other well-performed rat teratology studies which indicated that thiabendazole caused adverse developmental effects in rats only at higher maternally toxic doses. An ADI of 0.1 mg/kg bw was identified by applying a safety factor of 100 to the overall toxicological NOEL of 10 mg/kg bw/day. There is an adequate margin of safety between this ADI and the no-effect level for teratogenicity in mice (no teratogenicity at 120 mg/kg bw/day or less; teratogenic effects at 200 mg/kg bw/day or more). This ADI is identical to the ADI recommended by the 40th meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in June 1992 and was confirmed by the 48th meeting of JECFA in February 1997.

26. The JECFA toxicological evaluation cited data from a published study which proposed that in mammals thiabendazole is metabolised to 5-hydroxy-thiabendazole, 5-hydroxy-thiabendazole-glucuronide, 5-hydroxy-thiabendazole-sulphate, 4-hydroxy-thiabendazole, and a 2-acetylbenzimi-dazole derivative. According to JECFA, the total thiabendazole-related residues can be approximated by the sum of thiabendazole and 5-hydroxy-thiabendazole and its conjugates.

27. For the extension to include caprine species in Annex I the information summarised in the paragraphs below was taken into account.

28. There were no data, radiometric or otherwise, to determine the ratio of marker residue (the sum of thiabendazole and 5-hydroxythiabendazole) to total tissue residues in tissues of the target species.

29. Three sheep were dosed orally with thiabendazole. One animal was treated at a dose of 60 mg/kg bw and killed 4 hours after treatment; residues of thiabendazole in muscle, liver, kidney and heart fat were 380, 680, 4120 and 4400 µg/kg, respectively. One animal was treated at a dose of 82 mg/kg bw and killed 7 days after treatment; residues of thiabendazole in muscle, kidney and liver were 20, 30 and 40 µg/kg, respectively; no fat samples were analysed. One sheep was treated at a dose of 100 mg/kg bw and killed 7 days after treatment.; residues of thiabendazole in muscle, kidney, liver and heart fat were 20, 30, 90 and 40 µg/kg, respectively. This study did not meet the requirements of Volume 8 of the Rules Governing Medicinal Products in the European Community (data cited by JECFA).

30. Four sheep were dosed orally with 44 mg thiabendazole/kg bw and killed 1, 2, 3 and 4 days after dosing (1 per time point). Residues of thiabendazole + 5-hydroxy-thiabendazole in liver were 4680 µg/kg 1 day after dosing and 326, 825 and less than 50 µg/kg respectively at 2, 3, and 4 days after treatment. In muscle residues of thiabendazole + 5-hydroxythiabendazole were 580 µg/kg 1 day after treatment and less than 50 µg/kg at all later time points. This study did not meet the requirements of Volume 8 (data cited by JECFA).

31. Three calves (1 per group) were orally dosed with 11, 150 and 200 mg ^{14}C-thiabendazole/kg bw and slaughtered on days 34, 59 and 57 respectively after treatment. The total tissue residues (in ^{14}C-thiabendazole equivalents) were 1,500, 390 and 590 µg equivalents/kg, respectively in liver, 150, 110 and 130 µg equivalents/kg in kidney, 0, 130, and 160 µg equivalents/kg in muscle and 100, less than 70 and 180 µg equivalents/kg in fat. This study did not meet the requirements of Volume 8 (report cited by JECFA).

32. In a GLP study, calves were orally dosed with 75 mg thiabendazole/kg bw and then killed 1, 2 and 6 days after treatment (4 per time point). The mean residues of thiabendazole + 5-hydroxythiabendazole in tissues, determined by HPLC-fluorescence detection, 1, 2 and 6 days after treatment were: 472, 215, and 63 µg/kg in liver, 638, 97, and less than or equal to 50 µg/kg in kidney, 79, 50 and less than or equal to 28 µg/kg in muscle and 89, less than or equal to 50, less than or equal to 50 µg/kg in fat, respectively. The highest residues of thiabendazole + 5-hydroxythiabendazole were found in kidney samples (up to 1357 µg/kg) collected at the first timepoint. However, due to the persistence of high residue concentrations in the liver, it is a more appropriate target tissue for residue surveillance purposes than kidney.

33. Nine lactating cows were orally dosed with 0.5, 1.5 and 5 g of encapsulated thiabendazole daily for 28 consecutive days. Assuming that the cows weighed 500 kg, these doses would correspond to approximately 1, 3 and 10 mg/kg bw/day. Whole milk samples were collected at regular time intervals between -1 up to 56 days after the first daily dose. Two cows from each treatment group were killed at the end of the 28 day treatment period (study day 29). The remaining animals were slaughtered 28 days after the cessation of treatment (day 57) of the study. Highest residues of thiabendazole + 5-hydroxy-thiabendazole were found in kidney: 56 µg/kg (0.5 g dose), 270 µg/kg (1.5 g dose) and 466 µg/kg (5g dose) at the zero day withdrawal time and 30 µg/kg (0.5 g dose), 28 µg/kg (1.5 g dose) and 36 µg/kg (5.0g dose) 28 days after treatment. The other tissues could be ranked in order of highest marker residue content as follows: liver much greater than fat which in turn was greater than muscle (report cited by JECFA).

    During the 28-day dosing period the marker residue concentrations in milk samples ranged from 20 to 36 µg/kg in the 0.5 g (approximately 1 mg/kg bw) dosed animals, 33 to 162 µg/kg in the 1.5 g (approximately 3 mg/kg bw) dosed animals and 82 to 262 µg/kg in the 5.0 g (approximately 10 mg/kg bw) dosed cows (study cited by JECFA).

34. In a GLP study, 10 lactating (13.8 and 22.8 litres/day) cattle were orally dosed with 66 mg thiabendazole/kg bw. Whole milk samples from each cow were collected at 12 hour intervals between –12 hours and 84 hours timepoints with respect to dosing. The mean residues of thiabendazole + 5-hydroxythiabendazole in milk samples, determined by HPLC-fluorescence detection, were as follows: 6 µg/kg (-12 hours), 15 µg/kg (0 hours), 5175 µg/kg (12 hours), 3320 µg/kg (24 hours), 1089 µg/kg (36 hours), 364 µg/kg (48 hours), 304 µg/kg (60 hours), 117 µg/kg (72 hours) and 45 µg/kg (84 hours).

35. A validated analytical method for the determination of the marker residue in cattle tissues and milk, HPLC-fluorescence detection, was presented in the ISO 78/2 format. The limits of quantification were 50 µg/kg for thiabendazole and 50 µg/kg for 5-hydroxythiabendazole, for all tissues and for milk. This method should be applicable to caprine species and therefore from this aspect extrapolation to the tissues and caprine milk is possible.

36. The ratio of marker to total residues in cattle tissues and milk had not been determined and so a conservative estimate of marker to total residue ratio value of 0.5 was used. The available data suggest that this value is more realistically 0.9. This approach therefore afforded an extra margin of safety.

37. The MRLs proposed by the Committee are identical to those adopted by JECFA. Total consumer intake of residues arising from all crop use, preservative use and veterinary use was estimated by JECFA to be approximately 58% of the ADI. In this JECFA calculation, the veterinary use was assumed to represent less than 2%. However, JECFA took a less conservative approach for its consumer intake calculation than was deemed necessary by the Committee when taking into account all available data.

38. Ruminant species such as bovine, ovine and caprine species 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 goats were unlikely to show any significant differences in these parameters. Therefore, it was considered appropriate to recommend the extension of the MRLs so that that the same MRL values would apply to caprine species, including milk.

Conclusions and recommendation

Having considered that:

- an ADI of 0.1 mg/kg bw (i.e. 6000 µg/person) was previously established for thiabendazole,
- MRLs were previously established in bovine species; these MRLs would apply to caprine species,
- an analytical method for the monitoring of residues in tissues and milk of caprine species was available;

the Committee for Medicinal Products for Veterinary Use recommends the inclusion of thiabendazole 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
Thiabendazole	The sum of thiabendazole and 5-hydroxy-thiabendazole	Caprine	100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg 100 µg/kg	Muscle Fat Liver Kidney Milk	

Based on these MRLs, the theoretical daily intake of total thiabendazole-related residues in meat and milk corresponds to around 7% of the ADI. This would leave scope for the continued use of thiabendazole as a fungicide for crop protection and as a post-harvest preservative on stored fruits and potatoes.