



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

TILUDRONIC ACID, DISODIUM SALT

SUMMARY REPORT

1. Tiludronic acid as the disodium salt (disodium tiludronate), is a synthetic derivative of pyrophosphate belonging to the class of biphosphonates. This 4-chlorophenyl-thiomethylene biphosphonate is intended for use as an intravenous solution in horses to treat navicular disease, bone spavin and fetlock suspensory ligament enthesopathies. The recommended dose is 0.1 mg/kg bw per day for 10 days. It is also used in human medicine, in Paget's disease at a recommended therapeutic dose of 400 mg per person per day (6.6 mg/kg bw) for 3 months.
2. The primary target of its pharmacological action is considered to be osteoclasts. It inhibits the activity of the osteoclasts by promoting the inhibition of bone resorption as demonstrated in baboons receiving tiludronic acid as the disodium salt at oral doses of 0, 10, 28 and 80 mg/kg bw/day expressed as tiludronic acid for 6 months or for 1 year or in rats treated with oral doses of 50 and 200 mg/kg bw/day expressed in tiludronic acid for 6 months.
3. Tiludronic acid as the disodium salt has been shown to exert a dose-dependent inhibitory activity on bone resorption in a series of *in vivo* studies including thyroparathyroidectomized rats, calcergy induced rats, osteoporosis-induced rats, osteopenia-induced rats and arthritis-induced rats.

The doses used ranged from 0.5 to 50 mg/kg bw and from 3.125 to 200 mg/kg bw for the subcutaneous and oral routes, respectively. Tiludronic acid was administered as a single dose or repeatedly during a period varying from 3 days up to 6 months. The inhibition of bone resorption was assessed by measurement of the calcaemia, of the resorption of hydroxyapatite crystal plaques, of the bone density associated with calcium, phosphorus and hydroxyproline quantification. It was also assessed by its effect on osteopenia and on the polyarthritis index. From this set of pharmacological models, an oral pharmacological NOEL of 6.25 mg/kg bw based on the absence of activity in arthritis-induced rats could be retained. However, as tiludronic acid acts on bone physiology, other pharmacological targets corresponding to the sites of the pharmacological activity may have not been considered in those models, therefore the final statement on an overall pharmacological NOEL will be given after having taken into consideration the results of the long-term toxicity studies.

4. Effects on the central nervous system, on the gastrointestinal tract, on hydroelectrolytic balance and on inflammatory systems were also tested in a series of classical pharmacological tests in rats. No adverse effects on the central nervous system were reported up to single oral administrations of 400 mg/kg bw. Gastric emptying was significantly reduced after single oral administrations of 100 and 200 mg/kg bw of disodium tiludronate but without a dose relationship, and no effect was reported after a single oral administration of 25 mg/kg bw. No adverse effects on gastric secretion and intestinal transit were reported after single oral administration of doses up to 200 mg/kg bw. Disodium tiludronate had no anti-inflammatory properties on carrageenan-induced oedema when administered at doses of 400 and 10 mg/kg bw by oral or subcutaneous route, respectively. From these rat studies, an oral pharmacological NOEL of 25 mg/kg bw, expressed in tiludronic acid, can be derived from the gastrointestinal test.

5. Effects on the respiratory and cardiovascular systems were mainly studied in dogs. A single intraduodenal administration of 5 mg/kg bw of tiludronic acid as the disodium salt induced an increase in ventricular contractility and tidal volume.

In anaesthetised dogs, a dose of 10 mg/kg bw of tiludronic acid given as the disodium salt by continuous intravenous infusion can induce significant variations in the cardiovascular and respiratory parameters when administered in a short time, e.g. 10 and 60 minute-length. Adverse effects were limited to a reduction of the stroke volume and a decrease in the ventricular work when infused over a long time period, e.g. 120 minutes. No oral pharmacological NOEL could be derived from these dog studies.

6. Pharmacokinetic profiles of disodium tiludronate have been studied using ¹⁴C-disodium tiludronate after oral and intravenous doses in mice, rats, rabbits, dogs and monkeys.

Five minutes after a single intravenous administration of ¹⁴C-disodium tiludronate at doses of 12.5 mg/kg bw expressed in tiludronic acid in the different laboratory species, the radioactivity levels ranged from 40.13 (mice) to 155.16 (rabbits) mg equivalents tiludronic acid/l. Twenty-four hours after the intravenous administration, the concentrations were 0.10, 1.32, 1.43 and 3.69 mg equivalents tiludronic acid/l in mice, monkeys, rabbits and dogs, respectively. Within 144 hours, nearly the main fraction of intravenous administered dose was eliminated via urine, e.g. 37.32% in rabbits, 41.61% in rats and 46.67% in mice. Only a low fraction ranging from 3.48% (rabbits) to 7.81% (mice) was eliminated via faeces. About 50% of the dose administered were not eliminated within 144 hours.

Although the pharmacokinetic parameters were studied, only a rough estimate of the values of the different parameters could be made as the sensitivity of the analytical method was not sufficient to quantify the concentrations in the terminal phase of the disodium tiludronate depletion in plasma. In all species, the half-lives of elimination were quite long and estimated to be at least equal to 100 hours except for dogs (56 hours).

After single oral administrations of ¹⁴C-disodium tiludronate at a dose of 50 mg/kg bw expressed in tiludronic acid, the maximum concentrations in plasma appeared very rapidly (t_{max} at 0.5 to 1 hours) after dosing except in baboons (t_{max} at 4.5 hours). The levels ranged between 10 and 16 mg equivalents tiludronic acid/l in the species tested other than rabbits, which exhibited a maximum plasma of 3.5 mg equivalents tiludronic acid/l.

The oral availability was quite poor and estimated to be 1, 5.7 and 11% in rabbits, dogs and rats, respectively. It was slightly higher, about 15%, in mice and monkeys.

Within 144 hours, the major fraction of the oral radioactivity administered was recovered in faeces, approximately 80% in rabbits, rats, mice and monkeys. In urine, less than 5% of the oral radioactivity administered were recovered. In dogs, 67.38% and 11.54% of the oral dose administered were recovered in faeces and urine, respectively.

The characterisation and identification of metabolites in plasma and urine samples showed that the unchanged parent compound represented nearly the totality of the radioactivity (no percentage given). According to the species, 1 or 2 minor peaks resulted from a degradation reaction during the storage sample and/or during the extraction rather than a biotransformation of the parent compound were detected.

The influence on absorption of the food and the vehicle was tested in rats after single oral administrations of disodium tiludronate at a dose of 50 mg/kg bw, expressed in tiludronic acid. The best absorption conditions were obtained when the substance in water solution was administered to animals after an overnight fasting. When administered in water solution, in fed animals the highest concentrations of tiludronic acid in plasma were in the magnitude of 1 mg/l whereas in fasted animals they were close to 11 mg/l.

In rats, after single doses of ¹⁴C-disodium tiludronate in aqueous solution administered either by oral or intravenous routes at doses of 150 mg/kg bw or of 50 mg/kg bw respectively, expressed in tiludronic acid, the tissue distribution of the radioactivity was studied by whole body autoradiography and radioactivity was also quantified by liquid scintillation counting method. A selective tropism for all of the compact and spongy bone tissues as well as the hyaline cartilage was shown. During bone growth (14 to 28 days after administration), the accumulation of the radioactivity was always present in the calcified part and the osteogenesis area showed moderate radioactivity.

In rats given a single oral radiolabelled dose of ¹⁴C-disodium tiludronate at a dose of 50 mg/kg bw expressed in tiludronic acid, the levels in bone tissues ranged from 28.000 to 69.050 µg equivalents tiludronic acid/kg according to the type of bones, 1 hour after dosing. At 40 days, significant amounts of radioactivity were still measured: 11 980 to 29 980 and 18 020 to 19 290 µg/kg in femur and scapula, respectively. About 0.5% of the dose administered remained in the skeleton 40 days after the administration. At 1 hour post dosing, in cartilaginous tissues, the radioactivity levels ranged from 1870 to 15 090 µg equivalents tiludronic acid/kg according to their localisations. Then, the radioactivity decreased slowly to approximately 300 and 2000 µg equivalents tiludronic acid/kg in xyphoid process and trachea, respectively, at 40 days. In the other tissues, seven days after dosing, significant amounts of radioactivity were still found in the gastrointestinal walls of duodenum and ileum (290 to 3000 µg equivalents tiludronic acid/kg). In liver, kidney and spleen, 345, 535 and 260 µg equivalents tiludronic acid/kg were still measured.

7. In humans, approximately 90% of disodium tiludronate were bound to serum albumin and binding was linear in the concentration range 1 to 10 mg/l. In humans, after oral administration, an absolute oral bioavailability of approximately 6% has been reported with large inter- and intra-subject variability (assessed when disodium tiludronate was given formulated as a tablet containing lauryl sodium sulfate). The extent of absorption was significantly reduced when disodium tiludronate was administered within 2 hours after food or dairy product consumption.
8. Acute oral LD₅₀ values were close to 1000 and 560 mg/kg bw in mice and rats, respectively. Following intravenous administrations, the LD₅₀ values ranged from 100 to 150 mg/kg bw in both species. The main clinical signs consisted in cyanosis, prostration, respiratory and gastric disturbances, motor incoordination and seizures. The macroscopic examinations revealed lesions in kidney (pale cortical, medulla congestion), in gastrointestinal tract (congestion, haemorrhage) and in lungs (congestion).
9. The repeated dose toxicity of tiludronic acid as the disodium salt was investigated in mice, rats and baboons. Most of the studies have been carried out in fasted animals.

In an oral 13-week toxicity study, groups of mice (15 animals/sex/group) received, by gavage, tiludronic acid as the disodium salt in water solution at doses of 0, 42, 83 and 167 mg/kg bw/day expressed as tiludronic acid for 13 weeks. Mortalities were recorded at all doses. The most important findings were a nephrotoxicity associated with a renal tubular necrosis and a dose-related impact in the metaphyseal bony trabeculae of the femur. No NOEL could be derived from this study.

Tiludronic acid toxicity was investigated in rats following oral administrations for 5 and 13 weeks and for 6 months.

Four groups of rats (10 animals/sex) received by oral gavage, tiludronic acid, as the disodium salt, in 10% arabic gum at doses of 0, 50, 160 and 500 mg/kg bw/day expressed as tiludronic acid for 5 weeks. In the high dose group, a decrease in body weight as well as changes in electrocardiogram, biochemical and haematological parameters were recorded. In all the treated groups, mortalities were reported and the histology examination revealed renal tubulopathy and gastritis. No NOEL was retained.

In a second study, 5 groups of CD(SD)BR rats (20 animals/sex/group) received tiludronic acid, as the disodium salt, in water solution by oral gavage at doses of 0, 4.2, 10.4, 25 and 67 mg/kg bw/day expressed as tiludronic acid for 13 weeks. A significant increase in metaphyseal bony trabeculae of femur was noted in the 3 highest dose groups and a significant decrease in plasma calcium and phosphorus level was also reported. At 4.2 mg/kg bw, a significant decrease in plasma inorganic phosphorous was only noted in females at week 6. This effect was not reported at week 13. Therefore, a LOEL of 4.2 mg/kg bw/day was retained based on the variation of the level of phosphorous in plasma, which was considered related to the pharmacological activity of tiludronic acid.

In a third study, four groups of rats (30 animals/sex) received by oral route tiludronic acid as the disodium salt in 10% arabic gum at doses of 0, 12.5, 50 and 200 mg/kg bw/day expressed as tiludronic acid for 6 months. High levels of mortality were reported in all the treated groups (20, 21 and 50% at the terminal sacrifice in the 12.5, 50 and 200 mg/kg bw dose groups versus 6% in the control group). Although this high degree of mortality was attributable to improper gavage, the study was considered inadequate and no NOEL could be retained.

Two repeated oral toxicity studies were carried out in baboons. Tiludronic acid as the disodium salt in 10% arabic gum was administered at doses of 0, 10, 40, 80 and 160 mg/kg bw/day expressed as tiludronic acid, for 5 weeks in a first study and of 10, 28 and 80 mg/kg bw/day expressed as tiludronic acid, for 6 months. At interim and terminal sacrifices, renal tubulopathy, gastric erosions and lung congestion were recorded at doses higher or equal to 28 mg/kg bw. Although no adverse effect on blood chemistry, urinalysis, macroscopic and microscopic examinations were seen at 10 mg/kg bw, radiographic examination showed the presence of a well-defined opaque string located at the distal extremities of fore- and hind- limbs in the metaphyseal region beneath the epiphyseal cartilage. This increased bone density was barely visible after 3 months of treatment, but clearly apparent after 6 months. The width and the intensity of these strips were related to the doses administered. No NOEL could be derived from this study due to the pharmacologically activity of tiludronic acid. At the end of a 9-month reversibility period, most of changes recorded during the treatment period returned to normal.

10. Tolerance studies were performed in horses. Animals received tiludronic acid as the disodium salt at single intravenous doses ranging from 0.01 to 3 mg/kg bw or repeated doses of 0.1 mg/kg bw/day for 10 days, the recommended dose. At the therapeutic dose, out of all the 50 injections given, just 1 colic syndrome was observed in 1 animal after the 2nd injection. In 4 of the 5 animals, muscular hypotonia was observed after the 4th injection, increasing progressively until the eighth injection. No signs of local intolerance were reported.
11. Studies on reproduction were conducted in rats. In a one generation study, 4 groups of CD(SD)BR rats (34 animals/sex/group) received by oral route tiludronic acid as the disodium salt in distilled water at doses of 0, 8.3, 25 and 75 mg/kg bw/day expressed as tiludronic acid. Males were treated during 71 days before mating, throughout the mating period and up to termination at approximately week 15 of the study after females delivered. Females were dosed for 15 days before mating, during gestation and for those allowed to litter, during the lactation until termination after weaning to day 25 post-partum. Considering that it could not be excluded that the case of mortality reported at the highest dose could be related to the toxicity of tiludronic acid, a NOEL of 25 mg/kg bw/day for maternotoxicity was retained. No adverse effects on the offsprings were reported up to 75 mg/kg bw/day.

In a peri- and post-natal development study, 4 groups of 22 pregnant CD(SD)BR female rats received by oral route tiludronic acid as the disodium salt in distilled water at doses of 0, 8.3, 25 and 75 mg/kg bw/day expressed as tiludronic acid, from day 15 post-coitum to day 25 post-partum. Representative numbers of male and female offspring were selected and used to investigate the reproductive performance of the F1 generation. F1 animals were not treated. As 2 deaths occurred in the highest dose groups, a NOEL for maternotoxicity of 25 mg/kg bw/day was retained. At up to 75 mg/kg bw, tiludronic acid had no effect on development, behaviour or reproductive performances on untreated F1 generation.

No formal two-generation has been provided. However, considering the pharmacological activity of tiludronic acid, further information on reproduction was not requested as the 1-generation study did not reveal any particular adverse effects on reproduction.

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

In groups of 25 to 32 mated female NMRI BR mice, tiludronic acid as the disodium salt in distilled water was administered by oral route at doses of 0, 42, 125 and 375 mg/kg bw/day expressed as tiludronic acid, from day 6 to 15 post coitum. A evident maternotoxicity was reported at the highest dose. Some uncommon malformations in the paws (6/227 fetuses) were found at the highest dose and were considered to be incidental as only fetuses from one litter were affected. On the other hand, a treatment-related effect could not definitively be excluded in this concern. No adverse effects were noted in females and fetuses up to 125 mg/kg bw/day.

Four groups of 25 to 29 CD(SD)BR mated female rats received, by oral route, tiludronic acid as the disodium salt in 5% arabic gum at doses of 0, 42, 125 and 375 mg/kg bw/day, expressed as tiludronic acid, from day 6 to day 15 post coitum. At the highest dose, a statistically significant reduced mean body weight was reported during the treatment period. In rats, tiludronic acid is not teratogen. The doses of 125 mg/kg bw/day and 375 mg/kg bw/day can be retained as NOELs for maternotoxicity and embryotoxicity, respectively.

Two main teratogenicity studies were conducted in New Zealand white female rabbits. Tiludronic acid as the disodium salt was administered by oral route, from day 6 to day 18 of gestation. In the first study, tiludronic acid was given in 5% arabic gum solution at doses of 0, 42, 125 and 375 mg/kg bw/day, expressed as tiludronic acid and in the second study in water solution at doses of 0, 12.5, 50 and 200 mg/kg bw/day. A statistically significant decrease in the body weight was reported at the highest doses. A NOEL of 50 mg/kg bw/day was retained for maternotoxicity. The second study allows to conclude that the incidence of scoliosis found in the first study (2/102 fetuses and 1/79 fetuses in the 125 and 42 mg/kg bw treated groups, respectively) was incidental and not related to the pharmacological activity of tiludronic acid. Tiludronic acid was neither embryotoxic nor teratogenic in rabbits up to an oral dose of 200 mg/kg bw/day.

13. The mutagenic potential of tiludronic acid was tested in 7 *in vitro* test and 1 *in vivo* test. A battery of 4 tests to study gene mutation including *Salmonella typhimurium* reverse mutation assay, mitotic recombination in *Saccharomyces cerevisiae*, *Schizosaccharomyces pompe* forward mutation and gene mutation in mammalian cells (V 79 Chinese Hamsters Ovarian cells at the HGPRT locus) was used. One *in vitro* chromosomal aberration test was conducted using human lymphocytes. Tiludronic acid was also tested for DNA effects in an unscheduled DNA synthesis test using rat hepatocytes as primary cultures. An *in vivo* micronucleus test was conducted in mice after single oral administrations of tiludronic acid at doses up to 833 mg/kg bw expressed in tiludronic acid. As all these tests were carried out in the 1980s, the experimental design is not in accordance with the current requirements and some of them present deficiencies. All these tests gave negative results. One impurity of tiludronic acid, the sodium (4-chlorophenylsulfanyl)methylene diphosphonate, was negative in the Ames test.

It was concluded that tiludronic acid was devoid of mutagenic potential.

14. One carcinogenicity study and 1 long-term toxicity/carcinogenicity studies were conducted in mice and rats, respectively.

Groups of 112 CD-1(ICR)BR mice (56 animals/sex/group) received tiludronic acid as the disodium salt in distilled water by oral route at doses of 0, 8.3, 21 and 50 mg/kg bw/day, expressed as tiludronic acid, for 18 months. There was no significant statistical effect on incidence of any tumour type or on the total number of tumours. However, in all treated groups, an increased incidence in the bony trabeculae in sternum, femur and tibia was reported. This finding was considered as a pharmacological effect.

Five groups of 170 CD(SD)BR rats (85 animals/sex/group except 1 group with 80 animals) received tiludronic acid as the disodium salt in distilled water by oral route at doses of 0, 0, 4.2, 10.4 and 25 mg/kg bw/day expressed as tiludronic acid, for 104 weeks. Animals were fasted each day for 4 hours before treatment and 1 hour after. At week 104, the percentage of survivals in females was low (36, 34 and 33% in the 4.2, 10.4 and 25 mg/kg bw groups, respectively versus 44 to 40% in controls) whereas in males it was within the acceptable limits (42, 45 and 58% versus 58 to 53% in controls). There was no evidence of tumorigenic potential. A dose-related higher than control incidence of metaphyseal bony trabeculae in the femur and sternum was reported at the 2 highest doses at interim and terminal sacrifice in both sexes, and at 4.2 mg/kg bw at the interim sacrifice for females. In this long-term toxicity study, 4.2 mg/kg bw/day could be considered as a LOEL.

Tiludronic acid was not considered to have carcinogenic potential.

15. The effects of tiludronic acid on the immune system were tested in several experimental models including *in vitro* and *in vivo* tests. The 2 *in vitro* tests were murine non-specific lymphocyte proliferation and baboon non specific leukocyte proliferation. *In vivo*, different strains of mice were used to assess natural resistance to infections and the effects on specific humoral immunity and on delayed hypersensitivity. Two *ex vivo* studies for the evaluation of mitogen-induced lymphoproliferation in primates receiving long term treatment of tiludronic acid and arthritic rats were also carried out. Tiludronic acid had very weak pharmacological as well as toxic effects on the immune system of the species tested.

Two models were used to evaluate the antigenicity of tiludronic acid disodium salt in guinea pigs, the active systemic anaphylactic (ASA) model (animals were sensitised with the antigenic agent prior to the challenge) and a passive cutaneous anaphylactic (PCA) model (the animals received serum from sensitised animals by the intradermal route prior to challenge). No anaphylactic symptom was observed upon challenge by intravenous injection and the passive cutaneous anaphylactic reactions were negative.

Tiludronic acid did not induce any obvious antigenic or immunogenic effects in the tests systems used. However, due to the poor quality of the reports provided, this statement must be considered with caution.

16. Information on adverse effects in humans was available from clinical pharmacology studies and clinical experience conducted on more than 1600 subjects which were treated with tiludronic acid at daily doses of 100 to 1600 mg/person/day for 7 days to 6 months, 70% of subjects being treated with 400 mg/day, the recommended dose. The mean length of treatment was 3 months. The main adverse effects reported were gastrointestinal disturbances, which consisted in abdominal pain, nausea and diarrhoea. They were of low intensity, and the increase of the incidence was dose-dependent. The frequency of occurrence of gastrointestinal events was not modified by treatment duration. The most frequently reported symptoms in the Paget's patients were abdominal pain (10.9%), including primarily epigastric pain, nausea (7.2%), diarrhoea (5.7%), dyspepsia (3.6%), vomiting and flatulence (3.1%), headache (3%), asthenia (2.9%) and anorexia (2.1%).

The Periodic Safety Update from 1996 to 1999 indicate that the percentage of adverse effects reported in 29864 patients is quite low and less than 0.25%.

In a randomised double-blind study, thirty-eight menopausal women received for the first 6 months, tiludronic acid at 100 mg/person and during the second 6 months, all patients received the placebo. This 6-month oral treatment with tiludronic acid may counteract postmenopausal bone loss for at least a year by decreasing bone resorption.

In 2 additional clinical multicenter studies carried out in 2305 patients from Europe and in 2316 patients from USA, Canada and Australia and shortly reported in abstracts, tiludronic acid administered at doses of 50 and 200 mg intermittently (seven days per month) was not effective in reducing the incidence of vertebral fractures or increasing spinal bone mineral density. It was concluded that the doses and the regimens used were suboptimal.

No NOEL could be identified from the human data provided. However, 50 mg per person could be retained as a LOEL.

17. When the substance is known to have a specific target tissue/organ on which to exert its pharmacological activity, it is necessary to determine 2 NOELs, one for the toxicological effects and one for pharmacological effects. For tiludronic acid, the overall assessment of the safety profile clearly indicates that it is quite impossible to separate clearly the pharmacological effects from the toxicological ones. As tiludronic acid acts on bone resorption, variations on all different actors playing a role in bone physiology must be considered. That is the reason why the assessment of the full set of data available for this compound led to a single pharmacological-toxicological ADI.
18. From the human data, an oral LOEL of 50 mg per person, i.e. 0.833 mg/kg bw was retained from the clinical studies carried out in approximately 4600 patients. Applying a safety factor of 20, an ADI of 0.042 mg/kg bw (e.g. 2.5 mg per person) can be established.

From the set of data available for laboratory species, an ADI of 0.021 mg/kg bw/day (1.26 mg/person/day) was established based on an overall LOELs of 4.2 mg/kg derived from both the 13-week toxicity study in rats for effects on plasma phosphorus levels and from the long term/carcinogenicity in rats for effects on bone trabeculae. In establishing the ADI, a safety factor of 200 was applied.

Therefore, the most relevant ADI to consider is the ADI determined from the studies conducted in laboratory species.

19. In a non-radiometric study, three groups of 5 male horses (mean body weight of 391.9 kg, 3 to 6 years old) received a single intravenous administration of disodium tiludronate at doses equivalents to 0.05, 0.1 and 0.2 mg/kg bw of tiludronic acid (water solution).

In the 0.05 mg/kg bw group, the mean tiludronic acid concentrations in plasma declined from 0.604 mg/l, 5 minutes after dosing to 0.226 and 0.033 mg/l, 1 and 8 hours post dosing, respectively. They were below or equal to the limit of quantification (0.025 mg/l) in all animals at 24 hours after treatment.

In the 0.1 mg/kg bw dosed group, the mean tiludronic acid concentrations in plasma declined from 1.351 mg/l, 5 minutes after dosing to 0.496 and 0.068 mg/l, 1 and 8 hours post dosing, respectively. They were below or equal to the limit of quantification in all animals at 24 hours after treatment.

In the 0.2 mg/kg bw dosed group, the mean tiludronic acid concentrations in plasma declined from 2.414 mg/l, 5 minutes after dosing to 0.997 and 0.140 mg/l, 1 and 8 hours post dosing, respectively. They were below the limits of detection or quantification in all animals at 72 hours after treatment.

The half-life of elimination ($t_{1/2\beta}$) increased with the dose and was 3.13, 6.5, and 6.94 hours in the 0.05, 0.1 and 0.2 mg/kg bw groups, respectively. However, as the terminal phase of the depletion of tiludronic acid in plasma could not be correctly assessed due to the lack of the sensitivity of the analytical method, these figures should be taken with caution.

20. In a first radiometric study, 4 horses (2 males and 2 females) received by intravenous route a single dose of 0.1 mg/kg bw ^{14}C -tiludronic acid. In plasma, the mean level of total radioactivity declined from 1.375 mg equivalents tiludronic acid/l at 0.083 hours post dosing to 0.71, 0.014 and 0.06 mg equivalents tiludronic acid/l, 0.5 h, 24 hours and 48 hours post dosing, respectively. At 96 hours post treatment, radioactivity could still be measured in plasma (0.004 mg/l).

Within 96 hours, 24.45% and 47.53% of the intravenous dose were eliminated in males and females, respectively. The major fraction of the intravenous dose was eliminated via urine, accounting for 23.44 and 46.97% in males and females, respectively.

Ninety-six hours after the injection, the highest concentrations of radioactivity were found in bones. In samples taken within cancellous bone of the proximal metaphysis and in samples of the diaphysis (including cortical and cancellous bone), the mean levels were 705 and 213.25 μg equivalents tiludronic acid/kg, respectively. The bone concentrations were higher in males than in females, which could explain the slower excretion in males. In edible tissues, only a small quantity of radioactivity was found in muscle and fat (2 and 1.5 μg equivalents tiludronic acid/kg) whereas significant amounts were quantified in liver (39.50 $\mu\text{g}/\text{kg}$) and in kidney (231.25 $\mu\text{g}/\text{kg}$).

In horses, the majority of radioactivity (more than 81%) was bound to plasma proteins in both males and females at 2 and 24 hours post injection.

21. In a second radiometric study, 2 male and 2 female horses (264 kg bw, 2.5 to more than 5 years old) received daily intravenous administrations of ¹⁴C-tiludronic acid as the disodium salt according to the recommended therapeutic dosage, e.g. doses of 0.1 mg/kg bw, expressed as tiludronic acid, at 24 hour intervals, for 10 days. The total radioactivity in plasma was determined by liquid scintillation counting and the concentrations of the parent compound, tiludronic acid were determined by a HPLC method.

After the first injection, the total radioactivity levels declined from 1.275 mg equivalents tiludronic acid/l at 0.083 hours to 0.609 and 0.090 mg equivalents tiludronic acid/l, 1 and 8 hours post dosing, respectively. After the 10th injection, tiludronic acid concentrations declined from 1.293 mg equivalents tiludronic acid/l at 0.083 hours to 0.573 and 0.151 mg equivalents tiludronic acid/l, 1 and 8 h post dosing, respectively. During the treatment, the concentrations measured at 8 h after the injection increased slightly. The plasma radioactivity levels were 0.023 and 0.040 mg equivalents tiludronic acid/l, 24 hours after the first and the 10th injections, respectively.

After the first injection, the tiludronic acid concentrations declined from 0.916 mg/l at 0.083 hours to 0.460 and 0.064 mg/l, 1 and 8 hours post dosing, respectively. After the 10th injection, tiludronic acid concentrations declined from 0.909 mg/l at 0.083 hours to 0.504 and 0.110 mg/l, 1 and 8 hours post dosing, respectively. Twenty four hours after the first injection, tiludronic acid could not be quantified (less than 0.025 mg/l) in plasma whereas after the 10th injection, 0.040 mg/l could be quantified.

The mean ratio of the parent compound to the total radioactivity was estimated at 0.72.

The plasma pharmacokinetic parameters have been determined using a non compartmental analysis. After the first injection, the $t_{1/2\beta}$ and the clearance were 3.68 hours and 0.0462 l/h/kg bw, respectively. A slight increase in the area under the curve after treatment was reported when compared to that determined after the first injection (2.713 mg·h/l versus 2.120 mg·h/l). The ratio of these 2 area values lead to a figure of 1.31, illustrating the accumulation of tiludronic acid in body during the treatment.

22. The depletion of tiludronic acid in edible tissues of horses was investigated by using ¹⁴C-tiludronic acid. Four male and 4 female horses (264 kg bw, 2.5 to more than 5 years of age) received daily intravenous administrations ¹⁴C-tiludronic acid as the disodium salt at the recommended dosage, e.g. 0.1 mg/kg bw, expressed as tiludronic acid, at 24 hour intervals, for 10 days. Animals were sacrificed 12 and 48 hours after the last administration.

At 12 hours after the end of the treatment, the mean concentrations of radioactivity in muscle, fat, liver and kidney were 12.50, 14, 630 and 2569 µg equivalents tiludronic acid/kg, respectively; then they declined to 9.25, 11.50, 547.75 and 1739.50 µg equivalents tiludronic acid/kg, respectively, 48 hours post treatment.

23. Total drug derived residues in the standard food package amounted to approximately 195.90 µg at 12 hours and 145 µg at 48 hours post-dose. The major fraction of residues, were found in liver plus kidney at both sampling times and accounted for 191.45 and 142.5 µg equivalents tiludronic acid at 12 and 48 hours, respectively. These amounts represent approximately 97.5% of the amount of residue susceptible to be ingested by the consumer. As these 2 tissues are not normally considered as food commodities in the European Union, the amount of residues likely to be ingested after tiludronic acid treatment is negligible.

Conclusion and recommendation

Having considered the criteria laid down by the Committee for Veterinary Medicinal Products for the inclusion of substances in Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- tiludronic acid, as the disodium salt, is used in a small number of individual animals,
 - tiludronic acid, as the disodium salt, is intended for infrequent or non-regular treatments,
- and in addition that
- an ADI of 21 µg/kg (i.e. 1260 µg/person/day) was established,
 - the oral bioavailability of tiludronic acid in humans is low (approximately 6%),
 - residue depletion data were only provided for intravenous use,
 - at 12 hours after intravenous treatment the amount of residues likely to be ingested by consumers is far below the ADI and represents only a low fraction, approximately 15% of the ADI; 97% of this fraction of the residues being found in kidney and liver, the two equine tissues which are not considered as a food community in European community;

the Committee for Veterinary Medicinal Products concludes that there is no need to establish an MRL for tiludronic acid, as the disodium salt, and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Tiludronic acid, disodium salt	Equidae	For intravenous use only