The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

ALFACALCIDOL

SUMMARY REPORT

1. Alfacalcidol [synonym: 1α-hydroxycholecalciferol, 1α-hydroxyvitamin D_3, 1α-(OH)-D_3, 10-Secocholesta-5Z, 7E, 10 (19)-triene-1-α-3-ß-diol] is a synthetic analogue of vitamin D_3 used for the prevention of parturient paresis (milk fever) in dairy cows at the end of pregnancy. The recommended dose is 350 µg/animal by subcutaneous injection between 24 to 48 hours before calving. A second injection may be administered if calving does not occur within 24 to 48 hours of the first treatment but no more than two doses should be given. In human medicine, alfacalcidol is administered orally at maximum recommended daily doses 1 µg/day for adults (0.5 µg for elderly patients) and 0.05 µg/kg bw for children under 20 kg bw for the treatment of osteoporosis, renal osteodystrophy, hyperparathyroidism, neonatal hypocalcaemia, nutritional and malabsorptive rickets and osteomalacia.

2. The pharmacological properties of alfacalcidol were studied in different laboratory animals. Single intravenous injections at doses up to 400 µg/kg bw do not affect the respiratory and cardiovascular systems. No significant effects were observed on spontaneous locomotor activity, body temperature, sleep induced by pentobarbital, motor co-ordination, chemical or electro-shock induced convulsions and writhing behaviour in mice given oral doses of 5 to 250 µg/kg bw. There was no significant effect on avoidance response in rats, the electroencephalogram of rabbits or the mono- and polysynaptic spinal reflexes of cats orally dosed with 2.5 µg/kg bw. There was also no effect on autonomically-mediated contractions of the cat nictitating membrane preparation.

   In male rats, the oral administration of alfacalcidol at doses of 25 and 2.5 µg/kg bw, but not of 0.25 µg/kg bw, caused an increase in urinary calcium excretion, glomerular filtration rate, tubular reabsorption of phosphate and a decrease in urinary phosphate excretion. In dogs, the oral administration of 0.25 µg/kg bw of alfacalcidol for 3 weeks induced severe hypercalcaemia but no effect on renal function tests.

   In rats, after 10 days of treatment with 12.5 µg/kg bw, calcium concentration in the femur decreased and significant calcification was noted in soft tissues such as the kidney, intestine, aorta, heart and muscle. After 10 days of treatment with 2.5 µg/kg bw/day, calcification was only observed in the kidney.

   The exact mechanism whereby the inflow of calcium-ions into the extracellular fluid is increased is unclear. At the onset of lactation intestinal absorption of calcium is increased whereas bone resorption apparently does not contribute until 2 weeks *post partum*. Experimental findings suggest that, in contrast to vitamin D, 25-hydroxy-vitamin D_3, 1,25-dihydroxy-vitamin D_3 and alfacalcidol are able to initiate the bone resorption response prior to parturition.

3. The pharmacokinetics of alfacalcidol was studied in laboratory animals and target species. Intestinal absorption following oral administration of 24(S)-^{3}H-alfacalcidol (specific activity 3.8 Ci/mmol) was found to be 80% and 90% in normal and vitamin D deficient rats, respectively. The

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maximum plasma concentrations of alfacalcidol and 1,25-dihydroxy-vitamin D_3 were measured at 4 and 24 hours, respectively.

In dogs, a distribution half-life of 7 hours was observed following intravenous administration of 0.2 µg/kg bw of ^{3}H-alfacalcidol and the maximum plasma concentration of 1,25-dihydroxy-vitamin D_3 was 0.218 pmol/ml at 4 to 6 hours after dosing. Following oral administration of 0.2 µg/kg bw of ^{3}H-alfacalcidol, plasma levels of alfacalcidol and 1,25-dihydroxy-vitamin D_3 increased immediately with respective elimination half-lives ($t_{1/2ß}$) of 5 and 8 hours and C_{max} values of 0.265 and 0.328 pmol/ml at 4 hours after dosing. The C_{max} of 1,25-dihydroxy-vitamin D_3 following oral dosing was higher and was achieved more rapidly than following intravenous dosing. This was attributed to significant first-pass metabolism following oral administration.

Radioactivity following oral administration of 24(S)-^{3}H-alfacalcidol (specific activity 3.8 Ci/mmol) was distributed mainly to plasma, liver and small intestinal mucosa in normal and vitamin D deficient rats. Distribution to the cytosol and nuclear fractions of small intestinal mucosa were also apparent. The amount of radioactivity recovered in faeces over a period of 6 days corresponds to 39% and 49% of the administered dose after intravenous and oral dosing, respectively. A small percentage of non-volatile metabolites was excreted in urine. It is known that metabolites resulted from the metabolism of 1,25-dihydroxy-vitamin D_3 are either less potent or biologically inactive.

Following conversion of alfacalcidol to its active metabolite (1,25-dihydroxy-vitamin D_3) and transportation to its sites of action there is increased resorption of calcium and phosphorus and intestinal synthesis of calcium binding protein and calcium absorption. Success in the prevention of parturient paresis is achievable when either naturally occurring vitamin D_3 metabolites (25-hydroxyvitamin-D_3 and 1,25-dihydroxy-vitamin D_3) or the synthetic analogue alfacalcidol are used.

4. Acute dose toxicity studies were performed in mice, rats and dogs of both sexes.

   The oral LD_{50} values for mice and rats were 440 to 476 µg/kg bw and 340 to 720 µg/kg bw, respectively. The intravenous and subcutaneous LD_{50} values were 56 to 71 µg/kg and 85 to 96 µg/kg bw for mice and 56 to 101 µg/kg bw and 62 to 100 µg/kg bw for rats. Following intravenous dosing of mice there was depression of movement, reduced grooming and squinting at doses of 24 µg/kg bw or more. Depression of movement, reduced grooming, and excessive lacrimation were observed in rats dosed by the intravenous route. Necropsy findings included gastro-intestinal congestion, hydrothorax, hyperaemia and swelling of the lungs in survivors.

   Single dose toxicity was also investigated in Beagle dogs of both sexes. Signs of reaction after intravenous dosing included elevated pulse rate, head-shaking, scratching, lacrimation, increased water intake and reduced spontaneous movement. After oral dosing there were signs of gastro-intestinal disturbance, decreased spontaneous movement, salivation and lacrimation with signs of respiratory distress prior to death. Oral and intravenous LD_{50} values were estimated to be approximately 500 to 700 µg/kg bw and 300 to 400 µg/kg bw, respectively.

5. Several oral repeated-dose toxicity studies were performed in rats and dogs.

   A 30-day oral toxicity study in Wistar rats (groups of 25/sex/dose) receiving doses of ethanolic solutions dissolved in corn oil over the dose range 0.5 to 50 µg/kg bw/day was performed. Mortality, inhibited bodyweight gain, moderate leukocytosis, increased plasma calcium, total protein, total cholesterol, blood-urea nitrogen, and proteinuria occurred at doses of 12.5 µg/kg bw or higher. The main histological changes were necrosis of the intima of arterioles in cardiac muscle, voluntary muscle and the gastro-intestinal tract. The dosage rate of 0.5 µg/kg bw/day can be retained as a NOEL.

A 1-month oral toxicity study in dogs of both sexes treated with doses of 0, 0.04, 0.2, 1 and 10 µg/kg bw/day was carried out. None of the immature dogs treated at 10 µg/kg bw/day survived the 30-day treatment period. At 1 µg/kg bw/day and higher, bodyweight, food intake, and lymphocytes were decreased while urinary and plasma calcium concentrations, glutamic-oxaloacetic transaminase, alkaline phosphatase, blood-urea nitrogen, total bilirubin and neutrophils were increased. Necropsy findings included hydrothorax, pulmonary oedema, cardiac muscle degeneration, white discoloration in kidney and gastro-intestinal tract, intestinal haemorrhage and atrophy of the thymus and reproductive organs. The NOEL was considered to be 0.04 µg/kg bw/day.

A 3-month oral toxicity study in Wistar rats, dosed with 0, 0.02, 0.10, 0.5, 2.5 and 5 µg/kg bw/day was performed. Deaths occurred at 2.5 and 5 µg/kg bw. Other toxic manifestations included erythrocyte, leukocyte, lymphocytes, serum protein, albumin, glucose and potassium decreased while neutrophils, serum and urinary calcium increased. Ectopic calcification typical of hypercalcaemia was present in kidney and heart. The NOEL for these changes was 0.02 µg/kg bw/day.

A 6-month oral toxicity study in rats with doses of 0, 0.02, 0.1, 0.5 and 2.5 µg/kg bw/day was reported. A decrease in bodyweight gain and depression of food intake were apparent in the 2.5 and 0.5 µg/kg bw/day dose groups. An increase in serum and urinary calcium and inorganic phosphorus were present at doses of 0.1 µg/kg bw/day and higher. Histopathological studies revealed renal tubular degeneration, degeneration of cardiac muscle and blood vessel walls, atrophy of the medulla in the thymus gland and calcification of gastro-intestinal mucosa. A NOEL of 0.02 µg/kg bw/day was identified.

A chronic oral toxicity study in dogs was carried out for up to a 12-month period at doses of 0, 0.005, 0.02 and 0.08 µg/kg bw/day. Dogs treated with doses of 0.08 µg/kg bw/day revealed reduced weight gain and food intake, ataxia and emaciation as well as decreased in haematocrit, haemoglobin and erythrocyte counts. Increased serum calcium and inorganic phosphorus and blood-urea nitrogen were present. There were also effects on phenolsulphonphthalein excretion and glomerular filtration rate in the same dosage group. Histological changes (calcium deposition in the cavity and on the epithelial cells of renal tubules and atrophy of thymus cortex and medulla) were confined to the 0.08 µg/kg bw/day group. The dose level of 0.02 µg/kg bw/day can be retained as NOEL.

6. Tolerance studies were performed in bovine. Following single intramuscular injections of 0.5, 1, 1.5 and 3 mg of alfacalcidol in pairs of cows, no clinical or pathological evidence of hypervitaminosis D or soft tissue calcification was found. Acute iridocyclitis was reported in 3/3 non-pregnant cows 2 days after two intramuscular injections of 2 mg alfacalcidol in 5 ml sesame oil 7 days apart. Maximum plasma calcium (17.2 mg/100 ml) and phosphorus (13.3 mg/100 ml) values were recorded 3 to 5 days after treatment. Subendothelial calcifications in the left-side of the heart and the aorta were found at necropsy. lridocyclitis was also present in non-pregnant cows 2 days after the second of two intramuscular injections of 0.5 mg alfacalcidol.

   Another intramuscular tolerance study in 2 six-month old steers using a dose of 15 µg alfacalcidol/kg bw/day on four occasions at 7-day intervals was available. No clinical signs of iridocyclitis or any other lesions of the eyes were found at any time. After the first treatment serum activities of glutamic-oxaloacetic transaminase, glutamate dehydrogenase, alkaline phosphatase, blood-urea nitrogen, calcium and inorganic phosphate concentrations increased. *Post-mortem* examinations revealed metastatic calcification in the left atria endocardium, *bulbus aortae* and intima of the pulmonary artery, aorta, brachiocephalic trunk and carotid arteries.

   Finally, intramuscular doses of 420 µg/animal of alfacalcidol on three occasions at weekly intervals to non-pregnant dairy cows at the end of lactation produced an increase of calcium and phosphorus plasma concentrations and calcinosis of inner organs (aorta, heart, lung, kidney and uterus).

7. Data on the reproductive toxicity studies including fertility and peri and post-natal studies in rats were available. A fertility study was performed in Wistar rats by oral route administering doses of 0, 0.02, 0.1, 0.5 and 2.5 µg/kg bw/day to the males for at least 60 days and to female for 14 days prior to mating. Toxicity to the parent rats was evident at doses greater than or equal to 0.5 µg/kg bw/day and adverse effects upon mating performance, resulting in a reduced pregnancy rate, were present at these doses. Mating performance and fertility were unaffected at 0.02 and 0.1 µg/kg bw/day. There were no effects upon the numbers of *corpora lutea*, implantations and incidence of foetuses with external, visceral or skeletal anomalies. No effects were apparent on the postnatal growth and behavioural development of the offspring. The reproductive capacity and performance of the F1 generation was unaffected by the treatment of the dams. A NOEL of 0.1 µg/kg bw/day was retained.

   An evaluation of the potential of alfacalcidol to induce adverse effects when administered from day 17 of pregnancy to day 21 *post partum* was performed in pregnant Wistar rats given oral doses of 0, 0.02, 0.10. 0.5 and 2.5 µg/kg bw/day. Administration of 2.5 µg/kg bw/day suppressed bodyweight gain and depressed lactation. Bodyweight gain was slightly reduced at 0.5 µg/kg bw/day. However, treatment at doses up to 2.5 µg/kg bw/day did not adversely affect parturition or the nursing instinct of the dams. A decreased number of pups with reduced bodyweight were reared to weaning in the 2.5 µg/kg bw/day dose group. There was also a delay in the onset of vaginal opening in the female offspring obtained from dams treated at 2.5 µg/kg bw/day. No effects on behaviour in an open-field test and a conditioned avoidance test were apparent. The reproductive capacity of the F1 generation was unaffected by the treatment given to their dams. A NOEL of 0.5 µg/kg bw/day was retained.

8. Embryo- and foetotoxicity, including teratogenicity studies was studied in rabbits and rats. Wistar rats were treated with oral doses of 0, 0.02, 0.1, 0.5 and 2.5 µg/kg bw/day from days 7 to 17 of pregnancy. The dams treated at 2.5 µg/kg bw/day showed decreased weight gain and food consumption. A tendency for a reduction in the rate of bodyweight gain was also noted for the 0.5 µg/kg bw/day group. lncreased intra-uterine death rate and retardation of foetal growth and ossification occurred as secondary consequences to the severe maternal toxic effects at 2.5 µg/kg bw/day. A NOEL for reproductive parameters was identified as 0.1 µg/kg bw/day.

   The potential teratogenic effect was also studied in Himalayan rabbits that received oral doses of 0, 0.02, 0.08, 0.2 and 0.5 µg/kg bw/day on days 6 to 18 of pregnancy. One dam treated at 0.5 µg/kg bw/day died on day 17 of pregnancy after administration of 11 doses. The animal was emaciated, had diarrhoea and necropsy revealed gastric bleeding and perforation and surface discoloration of the heart and kidney. Diarrhoea and reduced faecal output were noted in the 0.08, 0.2 and 0.5 µg/kg bw/day groups and the incidence of abortion or premature birth in these groups was 1/13, 2/13 and 3/13, respectively. Effects on maternal bodyweight gain were seen in all dosage groups although there was no clear dosage-dependency when doses between 0.02 and 0.2 µg/kg bw/day were considered. There was an increased foetal resorption rate in the 0.08, 0.2 and 0.5 µg/kg bw/day dose groups although there was a lack of clear dosage-dependency. A NOEL for maternal adverse reactions was not defined in this study whereas the highest dose that did not adversely affect the outcome of pregnancy was 0.02 µg/kg bw/day.

9. The potential to induce reverse mutations in *Salmonella typhimurium strains* (TA98, TA100, TA1535, TA1538 and TA1537) was also examined using the spot test and plate incorporation methods devised by Ames. Solutions in dimethyl-sulfoxide of 0.25 to 250 µg/plate were applied in the spot test and 250 µg/plate was found to represent the limit of solubility in the plate incorporation method. There were no increases in the numbers of revertant colonies either in the presence or absence of metabolic activation.

   The potential of alfacalcidol to induce forward mutations at the thymidine kinase locus in cultured mouse lymphoma L5178Y cells in the absence and in the presence of metabolic activation was also assessed. Alfacalcidol did not induce any dose-related or statistically significant increases in the frequency of mutant colonies.

10. No carcinogenicity studies were performed.

11. The effects of short-term treatment with alfacalcidol on intestinal absorption of ^{47}Ca in healthy subjects and patients with chronic renal failure at doses varying from 0.14 to 5.4 µg/day were examined. A dose of 2.6 µg/day of alfacalcidol was required to increase intestinal absorption of ^{47}Ca in both groups. The half-time for urinary calcium to decrease to pre-treatment levels after stopping treatment were between 1.5 and 2.7 days. With long-term administration there was a progressive increase in intestinal absorption of ^{47}Ca in the patient group.

    In 9 subjects treated with 0.25, 1 and 2 µg alfacalcidol/day increase in serum calcium and phosphorus were present. A measurable response was seen at 0.25 µg/day and the mean maximum increases were 0.24 mmol/l for calcium and 0.27 mmol/l for phosphorus at 2 µg/day.

12. No specific data on immunotoxicity were provided. Tests for active and passive cutaneous anaphylaxis were performed in guinea pigs. Negative results were obtained when the test material was administered by the oral and intraperitoneal routes at 0.5 µg/kg bw.

13. A toxicological ADI of 0.002 µg/kg bw (i.e. 0.12 µg/person) may be calculated on the basis of the NOEL of 0.2 µg/kg bw/day in the 3-month repeated-dose oral toxicity study in rats and a safety factor of 100.

14. No information on the effects of alfacalcidol on human gut flora or on the microorganisms used for industrial food processing is available. However, no microbiological data are required for alfacalcidol.

15. Reports of the Scientific Committee for Food (EU) in 1992 indicate that intakes of 250 µg/day of vitamin D are harmful. The lowest level at which adverse effects appear is not known. Intakes of 50 µg/day appear to be safe. Thus, it would be prudent not to exceed 50 µg/day in habitual intake. An intake of 50 µg/day is equivalent to 50 times the recommended human therapeutic doses.

16. While no residue studies in milk were available, submission of such information was not considered necessary for this substance as the indication is restricted to the prevention of parturient paresis (milk fever) in dairy cows at the end of pregnancy. The possibility of residues in milk are therefore considered to be of no concern. Furthermore the biotransformation products of alfacalcidol are naturally occurring metabolites of vitamin D and, there are physiological mechanisms that will prevent hypercalcaemia in the extremely unlikely event that humans might consume residues in milk or tissues from treated. The safety margin afforded by the acceptable intake for vitamin D is large.

17. No routine analytical method for the determination of alfacalcidol in tissues of target animals was provided.

Conclusions and recommendation

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

- alfacalcidol is used in a small number of individual animals for infrequent and non-regular treatments,
- the treated animals are unlikely to be sent for slaughter during or immediately after treatment,
- alfacalcidol is rapidly absorbed, extensively metabolised and completed excreted,
- alfacalcidol is submitted to a significant first-pass metabolism following oral administration,
- the biotransformation products of alfacalcidol are naturally occurring metabolites of vitamin D;

The Committee considers that there is no need to establish an MRL for alfacalcidol and recommends its inclusion in Annex II to Council Regulation (EEC) No 2377/90 in accordance with the following table :

Pharmacologically active substance(s)	Animal species	Other provisions
Alfacalcidol	Bovine	For parturient cows only