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

EMEA/MRL/366/98-FINAL
March 1998

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

VITAMIN D

SUMMARY REPORT

1. The term vitamin D is used for a range of closely related steroid compounds which possess the property of preventing or curing vitamin D deficiency. These include alfacalcidol (1α-hydroxy-vitamin D_3), calcifediol (25-hydroxyvitamin D_3), calcitriol (1α,25 dihydroxy-vitamin D_3), cholecalciferol (vitamin D_3), dihydrotachysterol and ergocalciferol (vitamin D_2 or calciferol). In veterinary medicine, only vitamin D_3 is administered to food producing animals. Vitamin D_3 is given by oral, intramuscular and subcutaneous routes at the recommended posology of 500 to 2 000 IU/kg bw in cattle, sheep, horses, pigs, rabbits and chickens.

   Vitamin D_3 and vitamin D_2 are also used as feed additives at the maximum concentration of 2 000 to 5 000 IU/kg of complete feed for the individual domestic species and 10 000 IU/kg in milk replacers for calves and piglets.

2. Vitamin D activity is expressed in International Units (IU), 1 IU corresponding to 0.025 µg of vitamin D_2 or D_3, equivalent to 65 pmoles of vitamin D.

   Vitamin D_2 and D_3 have similar activities for humans and animals but for poultry vitamin D_3 is about 10 times more active than vitamin D_2.

   Cooking processes do not appear to affect the activity of vitamin D.

   Calcitriol and its synthetic analogue alfacalcidol have a more potent action and shorter half-lives than vitamins D_3 and D_2. Calcifediol, an intermediate metabolite has some action of its own but it also converted to the more potent calcitriol. Dihydrotachysterol has relatively weak antirachitic activity but its actions are faster in onset and less persistent than those of calciferols.

3. Extensive animal studies have shown that vitamin D is required for normal growth and development. In food producing species, the dietary requirements range from 125 to 1 000 IU/kg feed, i.e. 6 to 46 IU/kg bw/day.

4. These fat-soluble compounds are sometimes considered to be hormones or hormone precursors which are essential for the proper regulation of calcium and phosphorus homeostasis by enhancing their reabsorption by the proximal tubules of the kidneys and for bone mineralization.

5. Provitamins D are found in plants as ergosterol and in animals as 7-dehydrocholesterol. With exposure to ultraviolet light, these compounds are converted to vitamin D_2 and to vitamin D_3. Vitamin D is present in fish-liver oils, butter, eggs and liver.

6. In humans and animals, both vitamin D_2 and vitamin D_3 are rapidly absorbed from the small intestine and transported to the blood by the lymphatic system by a vitamin D-binding protein (a specific α-globulin).

   In liver, vitamin D_2 and D_3 are hydroxylated to calcifediol and 25-hydroxyergocalciferol, respectively. These compounds undergo further hydroxylation in the kidney to form the active form calcitriol or the inactive form 24,25-dihydroxy derivative. The hydroxylation of vitamin D is regulated by the parathyroid hormone and plasma concentrations of phosphorus.

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In ruminants, vitamin D and its metabolites are degraded in the rumen giving to ruminants a relative protection against hypervitaminosis D.

7. In rats after intravenous administration of 0.7 µg/kg bw of ^{3}H-calcifediol, calcifediol represented approximately 76% of the serum radioactivity 8 hours after the injection whereas vitamin D_3 and vitamin D esters accounted for 6.8% and 9.8%, respectively. In the urine collected during the first 8 hours after the injection, the more polar fractions calcifediol and calcitriol accounted for 96% and 2.6%, respectively. Eight hours post-injection, in kidney as well as in liver approximately 90% of the radioactivity was still in the form of calcifediol. Polar metabolites such as calcitriol and vitamin D_3 were also detected.

8. In pigs, after oral administration of feed supplemented by vitamin D_3 at a daily dose of 0, 90, 350 and 250 000 IU/animal for one month (doses in IU/kg bw and weight of the animals not stated), at the end of the supplementation the blood levels of vitamin D_3 were 0.26, 0.33, 0.52 and 2 337 IU/ml (0.0065, 0.008, 0.013 and 58.43 µg/ml). They then declined to reach, 4 weeks after the end of the treatment, levels of 0.28, 0.22, 0.35 and 2.04 IU/ml. Twelve weeks after withdrawal of the treatment, the blood vitamin D levels in the highest dose level group were 0.64 IU/ml (0.016 µg/ml).

   In sheep, a relationship between the amount of vitamin D_3 administered and plasma vitamin D_3 concentrations was demonstrated following intramuscular administrations of 3 different doses of vitamin D_3 (1 000; 10 000 and 100 000 IU/kg bw). After the intramuscular administration of 1 000 IU/kg bw, during the first six days after the administration, the plasma concentrations of vitamin D_3 ranged from 0.004 to 0.005 µg/ml.

   In another study carried out in sheep, it was shown that one day after a single administration of vitamin D_3 at a dose of 100 000 to 125 000 IU/kg bw (more than 50-fold the therapeutic dosage) given by oral or intramuscular routes, the plasma concentrations of vitamin D_3 were in the magnitude of 0.007 to 0.008 µg/ml. they then declined slowly to reach values of 0.004 to 0.005 µg/ml 15 days post dose.

   After a single intravenous administration of 2 million IU of vitamin D_3 per animal (40 000 to 50 000 IU/kg bw), plasma vitamin D_3 concentrations were 1.47, 0.3, and 0.04 µg/ml at 1, 5 and 20 days post dose, respectively.

   After a single intravenous administration of 2 million IU of calcifediol per animal (40 000 to 50 000 IU/kg bw), the calcifediol plasma concentrations were 0.60, 0.40 and 0.030 µg/ml at 1, 5, and 20 days post injection.

   In sheep, three days after a single intramuscular administration of 50 µCi of ^{3}H-labelled cholecalciferol, all plasma radioactivity was associated with calcifediol (concentrations in µg/ml not given).

   After a single intravenous administration of ^{3}H-labelled vitamin D_3 in chickens at a dose of 10 IU or 100 IU/animal (age and weight of the animals not indicated), the parent compound and eight metabolites of vitamin D_3 were separated in the extracts of small intestine collected 24 hours after the injection and three of them (21,25-dihydrocholecalciferol, 21,26-dihydrocholecalciferol and calcifediol) identified. However, no information about their relative ratio was reported.

   Large species variations in vitamin D_3 metabolism have been reported.

9. The oral LD_{50} values were 42 mg/kg bw in rats and in mice and 80 mg/kg bw in dogs. Anorexia, weakness, increases in serum calcium and in serum phosphorus levels, haemorrhage, necrosis and mineralisation in almost all organs were common toxic findings.

10. In a 28-day toxicity study, rats fed with either a standard diet or a low-calcium diet were given, by oral route, a synthetic analogue of calcitriol at doses of 0.02 and 2 µg/kg bw/day. The distribution and severity of renal pathologic changes were dose-related in both the standard and the low-calcium diet group but the changes found in the low-calcium diet group were reduced when compared to those of the standard diet group. The toxicity of this calcitriol analogue was reduced in rats fed by low-calcium diet. However, no NOEL can be retained from this study.

    In dogs, the oral administration of 500 and 1 000 µg/kg bw/day of vitamin D_2 for 7 to 21 days induced nephropathy associated with hypercalcaemia.

11. Tolerance in target species was studied in cattle, pigs and horses.

    In cattle, the daily administration of ergocalciferol by oral route at dose of 30 million IU for 7 days (corresponding to 60 000 IU/kg bw; 30-fold the recommended therapeutic dose) did not induce adverse effects. After longer treatment (10, 21 and 30 days), macroscopic lesions such as organ mineralisation were reported and these lesions were time-related in their extent and severity.

    In pigs, the oral administrations of vitamin D_3 at doses up to 625-fold the recommended dosage induced clinical signs (anorexia, polyuria). At 25-, 125- and 625-fold the recommended dosage, a decrease in parathyroid activity and an increase in plasma calcium levels were reported. No adverse effects were noted up to 5-fold the therapeutic dose.

    In horses, cases of hypervitaminosis D have been reported after weekly injection of 4 million IU for at least two months or after daily supplementation of 200 000 IU for several months (no information on the weight of animals).

12. In humans, a variety of forms and analogues of vitamin D are available and the choice of agent depends on the disorder or illness to treat. Doses from 0.25 µg (10 IU) up to 5 mg (200 000 IU) may be used in adults, but doses above 2.5 mg per day increase the risk of toxicity. Excessive intake of vitamin D leads to the development of hypercalcaemia and its associated symptoms including hypercalciuria, ectopic calcification and renal and cardiovascular damage. Individual tolerance to vitamin D varies considerably; infants and children are generally more susceptible. Doses of 60 000 IU per day or more can cause hypercalcaemia.

13. The daily requirements of vitamin D in adults are small and may be met mainly by exposure to sunlight and/or from the diet. A dietary intake of about 200 to 400 IU (5 to 10 µg) of vitamin is generally considered as adequate for healthy adults (United Kingdom dietary values, United States recommended dietary allowance).

14. Published data on the depletion studies of the vitamin D were available. Most of them were conducted in an insufficient number of animals at dosages much higher than the recommended dosage. Nevertheless, this information has been reported in order to show that significant amounts of vitamin D can be found in kidney, fat, liver and in milk after treatment.

15. In pigs, after oral administration of feed supplemented by vitamin D_3 at a daily dose of 0, 90, 350 and 250 000 IU/animal for one month (doses in mg/kg bw not given), at the end of the supplementation the concentrations of vitamin D_3 in liver were 90, 70, 210 and 27 100 IU/kg (2.25, 1.75, 5.25 and 677.5 µg/kg). In the highest dose level group, they then declined to reach 890, 150 and 90 IU/kg (22.25, 3.75, 2.25 µg/kg) at 4, 12 and 24 weeks after the end of the treatment. No information was available for the other edible tissues.

16. In sheep, after a single intramuscular administration of 50 µCi of ^{3}H-labelled calcifediol or of 50 µCi of ^{3}H-vitamin D_3, significant amounts of radioactivity were quantified in liver and kidney. However, as the results were given in decays per minute/g of tissue, no assessment of the levels in the edible tissues could be done.

In a second study, ewes received a single intramuscular dose of 20 000 to 25 000 IU/kg bw of vitamin D_3 (10-fold the therapeutic dosage). Groups of four animals were killed 1, 2, 3, 4 and 7 months post injection. One month after the injection, the concentrations of vitamin D_3 measured were close to 2.90, 5.40, 2.90 and 4.0 µg/kg in muscle, fat, liver and kidney. Seven months after treatment, significant amounts of vitamin D_3 were still found in kidney (1.60 µg/kg) and in fat (3.26 µg/kg).

In a third study, 22 days after a single administration of 100 000 to 125 000 IU/kg bw of vitamin D_3 (more than 50-fold the therapeutic dosage) by oral, intramuscular or intravenous routes, the concentrations of vitamin D_3 were in the magnitude of 3.40 to 4.96 µg/kg in liver, 3.97 to 5.06 µg/kg in kidney and 4.73 to 6.46 µg/kg in fat, the lowest value being reported after oral dosage.

17. Published data reported that vitamin D_2 and vitamin D_3 and traces of minor forms such as 22,23-dihydroergosterol (vitamin D_4) and 7-dehydrositosterol (vitamin D_5) might be found in milk. Only trace quantities of vitamin D are normally found in bovine milk.

    After oral administration of single large doses of vitamin D_3 (5 or 10 million IU/lactating animal; approximately 10 000 to 20 000 IU/kg bw), significant amounts of vitamin D_3 could be measured in milk: 40 to 120 IU/l and 63 to 280 IU/l (1 to 3 µg/l and 1.58 to 7 µg/l) for the low and high dose groups respectively, during the 10 first days after the treatment. Twenty-eight days post dose, no vitamin D_3 could be detected.

    After intravenous administration of single large doses of vitamin D_3 (5 or 10 million IU vitamin D_3 per animal, approximately 10 000 to 20 000 IU/kg bw), the concentrations of vitamin D_3 in milk were close to 100 IU/l and 360 IU/l (2.5 and 9 µg/l) for the low and high dose groups respectively, on the seventh day after dosing. Twenty-eight days post dose, vitamin D_3 could be detected in a few animals (20 to 90 IU/l; 0.5 to 2.25 µg/l).

18. In lactating ewes, after a single intravenous administration of 2 million IU vitamin D_3 per animal (40 000 to 50 000 IU/kg bw), vitamin D_3 concentrations in milk were 360, 200, and 40 µg/l at 1, 3 and 10 days post dose, respectively. By day 20 post injection, the milk vitamin D_3 levels had returned to the pre-injection level (2 to 3 µg/l).

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:

- vitamin D is an endogenously available substance,
- vitamin D is a normal component in the diet of humans and animals,
- vitamin D is used in veterinary medicine only for short-term therapy in individual animals only,
- the animals are unlikely to be sent for slaughter during or immediately after treatment,
- the variable levels of vitamin D naturally present in edible tissues of animals would undoubtedly make the establishment of MRLs and their surveillance impracticable;

the Committee concludes that there is no need to establish an MRL for vitamin D 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
Vitamin D	All food producing species	