

## Risk Assessment

## Vitamin D

### General information

#### Chemistry

Vitamin D refers to a group of fat-soluble seco-steroid compounds. Two nutritionally significant compounds are vitamin D<sub>2</sub> (ergocalciferol) and vitamin D<sub>3</sub> (cholecalciferol). Vitamin D<sub>3</sub> is metabolised to the active steroid hormone 1,25-dihydroxyvitamin D<sub>3</sub> by successive hydroxylations in the liver and kidney. Vitamin D<sub>2</sub> is metabolised to 1,25-dihydroxyvitamin D<sub>2</sub> by the same enzyme systems. One milligram of vitamin D is equivalent to 40,000 international units.

#### Natural occurrence

Vitamin D<sub>3</sub> (cholecalciferol) is produced photochemically from 7-dehydrocholesterol in the skin by exposure to sunlight or ultraviolet light. Vitamin D<sub>2</sub> is formed similarly from ergosterol in plants, fungi and lower life forms.

#### Occurrence in food, food supplements and medicines

Vitamin D is found in only a few foodstuffs, with fatty fish and fish oils, liver, milk and eggs being the main natural sources. In most industrialised countries, including the UK, processed milk, some powdered milks, margarine, breakfast cereals, bread and chocolate bars are fortified with vitamin D. Human milk contains low levels of vitamin D, but infant formula is fortified with 0.001-0.0025 mg/100 kcal.

Vitamin D is present in a range of food supplements (including fish oil products) and licensed medicines. Both vitamin D<sub>2</sub> and vitamin D<sub>3</sub> are used in food supplements, at levels up to 0.0125 mg per daily dose, and for food fortification.

#### Other sources of exposure

No data have been identified.

#### Recommended amounts

The establishment of recommended intakes of vitamin D is difficult because it is produced endogenously as a result of exposure to sunlight, which cannot readily be quantified. COMA has not set a RNI value for those individuals leading a normal lifestyle. For individuals confined indoors, pregnant and lactating women, an intake of 0.01 mg/day was recommended by COMA. RNIs of 0.0085 and 0.007 mg/day were recommended for infants aged 0-6 months and 6 months to 3 years respectively (COMA, 1998).

## Analysis of tissue levels and vitamin D status

The conventional marker of vitamin D status is plasma 25-hydroxyvitamin D. This marker is employed because it reflects the main storage form of precursor substrate for vitamin D, 1,25-dihydroxyvitamin D, the formation of which is under homeostatic control.

## Brief overview of non-nutritional beneficial effects

Vitamin D analogues have been reported to induce cell differentiation and reduce proliferation, and based on *in vitro* and epidemiological studies it has been suggested that vitamin D could protect against both prostate and colon cancer. This hypothesis was not commented on by COMA in its review of diet and cancer. It has been proposed that the anti-proliferative effect of vitamin D may be of benefit in the treatment of psoriasis (and treatment with the vitamin D analogue calcipotriol is of established benefit). There are also some claims that vitamin D may inhibit or stop the development of a number of autoimmune disorders, such as rheumatoid arthritis, and that it or its analogues may suppress transplant rejection.

## Function

Vitamin D is metabolised to the steroid hormone 1,25-dihydroxyvitamin D, a process which is promoted by parathyroid hormone (PTH). 1,25-Dihydroxyvitamin D regulates calcium and phosphate metabolism via three target tissues: kidney, small intestine and bone. In the kidney, 1,25-dihydroxyvitamin D regulates calcium transport in the proximal tubule; in the small intestine, it regulates calcium and phosphate uptake from the gut. 1,25-dihydroxyvitamin D is also involved in the maintenance of plasma calcium levels via bone resorption and formation. 1,25-dihydroxyvitamin D regulates the synthesis of PTH by a negative feedback mechanism.

Vitamin D<sub>2</sub> and D<sub>3</sub> are generally assumed to have equal levels of efficacy in humans, although recent data suggest that vitamin D<sub>3</sub> may be more efficient at increasing serum 25-hydroxyvitamin D levels (Trang *et al.*, 1998).

## Deficiency

Prolonged vitamin D deficiency in infants and children results in rickets. In adults, vitamin D deficiency results in osteomalacia, the clinical symptoms of which include skeletal pain and muscle weakness and pathological fractures. Less severe vitamin D deficiency (usually referred to as vitamin D insufficiency) is associated with secondary hyperparathyroidism and increased bone loss, leading to high risk of fractures.

Groups such as black, Asian, institutionalised older people, and those who habitually cover the skin may form less vitamin D endogenously as a result of exposure to sunlight, and are vulnerable to vitamin D deficiency. Individuals with a decreased capacity for intestinal absorption of vitamin D, for example following partial gastrectomy, are also at increased risk of vitamin D deficiency, as are patients with liver, renal and cardiopulmonary diseases.

### Interactions

The cytotoxic agent actinomycin and imidazole antifungal agents interfere with vitamin D activity by inhibiting the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D by the kidney enzyme, 25-hydroxyvitamin D-1-hydroxylase. Lead has also been reported to inhibit vitamin D synthesis. Some anticonvulsant drugs may interfere with hepatic metabolism of vitamin D and so raise requirements.

### Absorption and bioavailability

Vitamin D is absorbed from the small intestine as bile salt-dependent micelles and circulated in the body via the lymph. Absorption of polar derivatives, such as 25-hydroxyvitamin D, is more efficient and less dependent on bile salts. These polar derivatives are generally not present in any significant amount in food or food supplements, although small amounts of 25-hydroxyvitamin D are found in meat and breast milk.

### Distribution and metabolism

There is substantial storage of vitamin D in adipose tissue. Vitamin D in plasma is bound to vitamin D-binding protein and transported to the liver where it undergoes 25-hydroxylation and is released into the circulation bound to  $\alpha$ -2 globulin. Additional 1-hydroxylation then occurs in the kidney, producing the active form of the vitamin. This reaction is regulated by parathyroid hormone (PTH), which is secreted in response to low plasma calcium levels.

### Excretion

Vitamin D is principally excreted in the bile. It is also metabolised to water-soluble metabolites, such as calcitroic acid, and excreted in the urine.

## Toxicity

### Human data

Excessive vitamin D intake may lead to hypercalcaemia and hypercalciuria. Vitamin D promotes the absorption of calcium and the resorption of bone resulting in the deposition of calcium in soft tissues, diffuse demineralisation of bones and irreversible renal and cardiovascular toxicity. Patients with sarcoidosis are abnormally sensitive to vitamin D, due to uncontrolled conversion of the vitamin to its active form in the granulomatous tissue. Although the condition is uncommon, it would be a potential hazard if affected individuals were to take supplementary vitamin D.

One study has suggested that moderate levels (0.025-0.050 mg/day) of vitamin D may enhance renal stone formation in susceptible individuals.

Data on the comparative toxicity of vitamin D<sub>2</sub> and D<sub>3</sub> in humans are lacking.

### Supplementation trials

A number of supplementation trials have reported no adverse effects at intakes of 0.010 – 0.045 mg vitamin D/day. The effects at higher doses have been more variable. For example, in a study in which 63 older individuals (females over 60 years, males over 65 years) were given 0.05 mg/day supplementary vitamin D (form unspecified) for 6 months, 2 subjects developed hypercalcaemia (Johnson *et al.*, 1980). However, in a 5 month study in which 61 younger adults (mean age, 41 years) were given 0.10 mg/day supplementary vitamin D<sub>3</sub>, serum calcium levels were not significantly increased (Vieth *et al.*, 2001). The difference may reflect differing prior states with hyperparathyroidism or hypovitaminosis D possibly occurring in the elderly.

### Animal data

In animals, excess vitamin D causes hypercalcaemia, resulting in deposition of calcium in soft tissues and bone demineralisation, anorexia, weight loss, anaemia and weakness.

In studies in rhesus monkeys, vitamin D<sub>3</sub> was shown to be significantly more toxic than vitamin D<sub>2</sub>. The authors noted that although less toxic, vitamin D<sub>2</sub> was still functional in this species.

Excess vitamin D<sub>2</sub> during gestation in rabbits led to decreased foetal viability, an increased number of abortions, and supra-auricular lesions in the offspring. High doses of vitamin D appear to affect maternal calcium, phosphate and cholesterol homeostasis and neonatal calcium homeostasis. In rodents, administration of high levels of vitamin D<sub>2</sub> during gestation resulted in retarded foetal and placental growth, loss of ossification of foetal bones and foetal skeletal degeneration, resulting particularly in facial malformations.

### Carcinogenicity and genotoxicity

There are no data relating to the carcinogenicity of vitamin D. No *in vivo* or *in vitro* genotoxicity data have been identified.

### Mechanism of toxicity

Vitamin D is involved in calcium metabolism and can increase calcium uptake from the gut, reabsorption of calcium from the kidney and resorption of calcium from bone. Such responses are not well characterised.

### Genetic variations

Children born with the rare autosomal recessive condition, vitamin D dependency rickets Type II, are vulnerable to rickets and alopecia. The condition is characterised by high circulating levels of 1,25-dihydroxyvitamin D<sub>3</sub> and functionless vitamin D receptors. Such individuals would be less susceptible to high intakes of vitamin D.

The gene coding for the vitamin D receptor has been reported to be polymorphic, and sensitivity to vitamin D has been shown to vary depending on genotype.

### Dose response characterisation

Due to the unknown contribution of vitamin D formed through exposure to sunlight, the dose response relationship of vitamin D and hypercalcaemia or hypercalciuria is difficult to determine in humans. Human supplementation studies using less than 0.02 mg/day were not associated with adverse effects, whereas adverse effects have been reported at higher levels of intake in some studies. The majority of studies have reported significant changes at supplementation levels of approximately 0.10 mg/day or greater.

A problem in characterisation arises from the difficulty in ascertaining whether a particular serum calcium level on vitamin D supplementation does or does not indicate normality. In deficient individuals serum calcium levels would be expected to rise less than in those initially replete. Such a situation could prevail in older people and in Asian populations.

### Vulnerable groups

Infants are at risk of developing hypervitaminosis D; hypercalcaemia has been reported at vitamin D intakes of 0.050 mg/day and above (see later). Adults with disease states such as sarcoidosis, *Mycobacterium* infections and idiopathic hypercalciuria and hypercalcaemia more vulnerable to hypercalcaemia resulting from moderate vitamin D intakes ( $> 0.025$  mg/day).

## Studies of particular importance in the risk assessment

(For full review see <http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers> or the enclosed CD).

### Human data

*Johnson et al., 1980*

A randomised, double-blind, placebo-controlled clinical trial of dietary supplementation with 0.05 mg vitamin D per day (form unspecified) for six months was carried out in females over 60 and males over 65 years of age ( $n = 63$ ). Serum calcium levels were significantly raised in the vitamin D-treated group and two of the subjects developed hypercalcaemia (serum calcium  $> 2.75$  mmol/L).

*Narang et al., 1984*

A clinical trial of vitamin D supplementation (form unspecified) was carried out in healthy subjects aged 21-60 years and patients with pulmonary tuberculosis. Groups were given 0.01, 0.02, 0.03, 0.06 and 0.095 mg vitamin D per day for 3 months. In the healthy subjects ( $n = 30$ ), serum calcium levels were significantly raised in the 0.06 and 0.095 mg/day groups, although levels only exceeded the normal range ( $> 2.75$  mmol/L) in the 0.095 mg/day group. Serum calcium levels were non-significantly raised in the 0.01, 0.02 and 0.03 mg/day groups.

*Honkanen et al., 1990*

This randomised clinical trial of dietary supplementation with 0.045 mg vitamin D<sub>3</sub> and 1558 mg calcium per day was performed in 52 independently-living and institutionalised elderly subjects treated for 11 weeks (27 controls, 25 treated). Serum calcium, 25-hydroxyvitamin D and creatinine levels were measured before and after the trial period. Serum calcium and creatinine levels did not significantly

differ in control or treated groups after the trial period, and were not outside the normal ranges in any individuals.

*Chapuy et al., 1992*

The effect of supplementation for 18 months with 0.020 mg/day vitamin D<sub>3</sub>, together with 1200 mg/day calcium, on hip fracture incidence was investigated in this randomised, placebo-controlled study in healthy women (mean age 84 years). Of the 1634 subjects who received the active supplements, 877 completed the study. However, the rate of dropout from the study was similar in both treated and placebo groups and there were no significant differences in the reasons reported for withdrawal (death, non-compliance, inability to walk during the study, loss to follow-up, intercurrent illness, gastrointestinal effects). Serum calcium levels were measured every 6 months and one subject in the treated group withdrew from the study due to mild hypercalcaemia (serum calcium 2.8 mmol/L), which was reported to be due to primary hyperparathyroidism. No other subjects had hypercalcaemia at any point during to the study and it was reported that no subjects developed renal calculi.

*Jacobus et al., 1992*

A retrospective study was carried out on eight US patients with hypervitaminosis D, thought to be caused by consumption of milk excessively fortified with vitamin D<sub>3</sub>. Serum vitamin D<sub>3</sub> concentrations varied considerably in these patients. A proposed explanation for this was that apparently the fortification of the milk was sporadic and only at times in excess. All patients had elevated serum 25-hydroxyvitamin D levels, with 6/8 having elevated serum vitamin D<sub>3</sub> concentrations. Seven of the eight patients had hypercalcaemia. All patients drank locally produced milk (118-710 mL/day) which contained vitamin D levels ranging from undetectable to 6.1 mg/L. The recommended level for milk fortification in the US is 0.01055 mg/L.

*Dawson-Hughes et al., 1997*

A randomised double-blind placebo-controlled clinical trial investigated dietary supplementation with 0.0175 mg vitamin D<sub>3</sub> and 500 mg calcium per day for 3 years in 389 subjects of 65 years or older. Although primarily a study of effects on bone mineral density, serum calcium and creatinine were monitored. There was a small but significant increase in serum calcium levels over the lifetime of the study in the treated group, but levels were within the normal range. One treated subject withdrew from the trial due to hypercalciuria.

*Vieth et al., 2001*

The safety of supplementation with 0.10 mg/day vitamin D<sub>3</sub> for 2 to 5 months was investigated in healthy men and women with a mean age of 41 years. The number of treated individuals with hypercalciuria was not significantly different to the number of untreated controls with hypercalciuria, although the study was of low statistical power to detect such differences. There was no evidence of hypercalcaemia; mean serum calcium concentrations were within the normal range (2.2-2.6 mmol/L) in all subjects.

## Animal data

*Haschek et al., 1977*

Pigs received standard diets that had optimal calcium and phosphate and provided intakes of vitamin D<sub>3</sub> of 0.00132 mg/kg bw/day for one week following weaning. They were injected with <sup>45</sup>Ca and, 3 days

later, divided into two groups, one of which received standard diet (control) and one of which received diet providing 0.825 mg/kg bw/day vitamin D<sub>3</sub>. Two pigs from each group were sacrificed 1, 2, 3, 4, 7 and 14 days after division into the two treatment groups. Pigs fed the high level of vitamin D lost weight; anorexia, weakness, rough hair coat and laboured breathing were also observed. In the treated group, hypercalcaemia began at 12 hours after starting the high vitamin D diet, and progressed rapidly after two days. Interpretation of the radioisotope studies indicated that bone was the primary source of the increased plasma calcium, since food intake and, therefore calcium absorption from the gut, was depressed. Calcium was released at a rapid rate from pre-labelled bone undergoing breakdown.

*Chineme et al., 1977*

Pigs were fed diets providing vitamin D<sub>3</sub> intakes of approximately 0.00132, 0.0066, 0.033 or 0.165 mg/kg bw/day. The resulting hypercalcaemia, seen in the upper two dose groups, was thought to result primarily from intestinal absorption of calcium, not from bone loss and tissue degeneration, as hypercalcaemia *per se* was not always associated with soft tissue calcification in pigs.

## Exposure assessment

Total exposure/intake:

Food                                      Mean: 0.003 mg/day  
     97.5<sup>th</sup> percentile: 0.009 mg/day (from 1986/87 NDNS)

Supplements                            Up to 0.0125 mg (Annex 4; OTC, 2001)

Estimated maximum intake    0.009 + 0.0125 = 0.022 mg/day

No potential high intake groups have been identified.

## Risk assessment

Excess vitamin D may lead to hypercalcaemia and hypercalciuria. Hypercalcaemia results in the deposition of calcium in soft tissues, diffuse demineralisation of bones and irreversible renal and cardiovascular toxicity. Moderate levels of vitamin D intake may enhance renal stone formation in predisposed individuals. It has been suggested that excess vitamin D may be linked to heart disease, but there is limited evidence for this.

Data are available from a range of human supplementation studies, but the levels of vitamin D intake at which hypercalcaemia or hypercalciuria occurs vary between studies. Likely reasons for this include differences in populations studied; for example, several of the studies are in older people, a group vulnerable to vitamin D deficiency, while other studies are in younger adults, who are not likely to be vitamin D deficient. Individuals and groups are also likely to differ in their exposure to vitamin D sources other than supplementation, such as consumption of vitamin D-fortified foods and through exposure to the sun.

Excess vitamin D during gestation in rats and rabbits led to a number of adverse reproductive effects.

## ESTABLISHMENT OF GUIDANCE LEVEL

A safe upper level suitable for long-term intake by the whole population cannot be established based on the available data from studies in humans or animals. The human data are adequate to provide guidance. High intakes of vitamin D are associated with elevated levels of calcium, which in turn lead to soft tissue calcification, demineralisation of bones, and renal and cardiovascular toxicity. The effect is apparent in both human case reports and in animal studies.

Assessing the risk of toxicity in man is complicated. On the one hand, significant numbers of the UK population, notably older people and Asian populations not exposed to sunlight, may ordinarily be at risk of deficiency. By contrast, apart from the rare individuals with granulomatous diseases, who are abnormally sensitive to the effects of vitamin D, there are others, notably older people, who have a high prevalence of primary hyperparathyroidism. Others, with tertiary hyperparathyroidism due to prolonged vitamin D deficiency, may show falsely low serum calcium levels in response to vitamin D. There is therefore a particular need for large studies covering all sections of the population. These are generally lacking.

The highest level of vitamin D supplementation at which no effect on calcium was observed was 0.10 mg/day, reported by Vieth *et al.* (2001). This was from a 5 month supplementation study of vitamin D<sub>3</sub> in 63 adults aged 23 – 56 years. In contrast, the lowest level of vitamin D at which effects have been observed was 0.05 mg/day (Johnson *et al.*, 1980) from a 6 month study of supplementation with vitamin D (of unspecified form) in females above the age of 60 and males above the age of 65. Two out of the 63 subjects developed hypercalcaemia (serum calcium > 2.75 mmol/L). The reason for the difference is not known but may be due to difference in other sources of vitamin D exposure or in the study populations.

Taking the evidence as a whole, long-term exposures of up to 0.025 mg/day vitamin D appear to be well-tolerated and may be necessary to prevent deficiency in some groups. Higher levels (for example, 0.045 mg/day; Honkanen *et al.*, 1990) may be tolerated without adverse effects over the short-term under medical supervision and may be necessary to correct a deficiency. The use of an uncertainty factor is not appropriate because the value is derived from an overview of a number of human studies which measured sensitive biochemical markers of calcium homeostasis.

For guidance purposes only, a level of 0.025 mg/day supplementary vitamin D would not be expected to cause adverse effects in the general population. This is equivalent to 0.0004 mg/kg bw/day for a 60 kg adult. Due to the difficulties in assessing total vitamin D exposure, an estimate for total intake has not been provided. Such an intake, or more, might well be required under medical supervision in managing overt or occult deficiency states. It should be noted that scaling on a body weight basis to children and infants may not be appropriate for vitamin D as it may lead to the recommended intake for an infant not being met.

## References

- Chapuy, M.C., Arlot, M.E., Duboeuf, F., Brun, J., Crouzet, B., Arnaud, S., Delmas, P.D., Meunier, P.J. (1992) Vitamin D<sub>3</sub> and calcium to prevent hip fractures in elderly women. *New England Journal of Medicine* **327**, 1637-1642.
- Chineme, C.N., Krook, L., Pond, W.G. (1976) Bone pathology in hypervitaminosis D: an experimental study in young pigs. *Cornell Veterinary Medicine* **66**, 387-412.
- COMA (1998). Nutrition and Bone Health: with particular reference to calcium and vitamin D. Report of the Subgroup on Bone Health, Working Group on the Nutritional Status of the Population, Committee on Medical Aspects of Food and Nutrition Policy. The Stationery Office, London.
- Dawson-Hughes, B., Harris, S.S., Krall, E.A., Dallal, G.E. (1997) Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *New England Journal of Medicine* **337**, 670-676.
- Haschek, W.M., Krook, L., Kallfelz, F.A., Pond, W.G. (1977) Vitamin D toxicity. Initial site and mode of action. *Cornell Veterinary Medicine* **68**, 324-364.
- Honkanen, R., Alhava, E., Parviainen, M., Talasniemi, A., Mönkkönen, R. (1990) The necessity and safety of calcium and vitamin D in the elderly. *Journal of the American Geriatrics Society* **38**, 862-866.
- Jacobus, C.H., Holick, M.F., Shao, Q., Chen, T.C., Holm, I.A., Kolodny, J.M., Fuleihan, G.E., Seely, E.W. (1992) Hypervitaminosis D associated with drinking milk. *New England Journal of Medicine* **326** (18), 1173-1177.
- Johnson, K.R., Jobber, J., Stonawski, B.J. (1980) Prophylactic vitamin D in the elderly. *Age and Ageing* **9**, 121-127.
- Narang, N.K., Gupta, R.C., Jain, M.K. (1984) Role of vitamin D in pulmonary tuberculosis. *Journal of the Association of Physicians of India* **32**, 185-188.
- Trang, H.M., Cole, D.E.C., Rubin, L.A., Pierratos, A., Siu, S., Vieth, R. (1998) Evidence that vitamin D<sub>3</sub> increases serum 25-hydroxyvitamin D more efficiently than does vitamin D<sub>2</sub>. *American Journal of Clinical Nutrition* **68**, 854-858.
- Vieth, R., Chan, P-C. R., MacFarlane, G.D. (2001) Efficacy and safety of vitamin D<sub>3</sub> intake exceeding the lowest observed adverse effect level. *American Journal of Clinical Nutrition* **73**, 288-94.