COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Statement on adverse effects of high levels of vitamin D.

Introduction

1. At the request of the Department of Health, the Scientific Advisory Committee on Nutrition (SACN) is reviewing dietary reference values for vitamin D, and as part of this process, the COT was asked to advise on the possible adverse effects of high vitamin D intakes. Such intakes could be regular and long-term, or from single or occasional doses of vitamin D supplements at higher levels. Advice was also sought on population groups which might be unusually vulnerable to adverse effects of vitamin D.

Previous assessments

2. The safety of high intakes of vitamin D has been considered previously by a number of expert bodies. In a 1991 report to the then Department of Health and Social Security, the Committee on Medical Aspects of Food and Nutrition Policy (COMA) (the predecessor of SACN) established dietary reference values for a range of nutrients, including vitamin D. COMA briefly considered high intakes; they did not specify an upper level for consumption, but noted that infants were the population group that was most susceptible to hypervitaminosis D (COMA, 1991). COMA also cited a report by Markestad *et al*., (1987) that mild hypercalcaemia had occurred in infants at vitamin D intakes of 50 µg/day or 15,000 µg every 3-5 months[1].

3. In 2002, the EU Scientific Committee on Food (SCF) established a Tolerable Upper Level (TUL)[2] of 50 µg/day for adults (SCF, 2002). In 2003, the UK Expert Group on Vitamins and Minerals (EVM) considered that there was insufficient information to establish a Safe Upper Level (SUL) for vitamin D but noted that for guidance purpose only, intakes of 25 µg/day supplementary vitamin D would not be expected to result in adverse health effects (EVM, 2003).

[1] Quantities of vitamin D can also be expressed as International Units (IU); 1µg is equivalent to 40 IU.
[2] SCF and EFSA have set TULs, IOM set ULs and EVM SULs. Although the terminology is different, the values represent a daily intake which, if consumed over a life time, would not be expected to result in adverse effects.

4. In 2011, an extensive review of vitamin D was undertaken by the US Institute of Medicine (IOM) Food and Nutrition Board (IOM, 2011), which established an Upper Level (UL) of 100 µg/day vitamin D for adults; this was an increase from the upper level of 50 µg/day previously recommended (IOM,1997). The IOM noted the paucity of long-term studies investigating the effects of vitamin D intakes above 250 µg per day or of maintaining serum 25-hydroxyvitamin D above 250 nmol/L. However, the IOM stated that it was unlikely that symptoms of toxicity would be observed at intakes below 250 µg/day, whereas adverse effects would be observed from consumption at or above 1250 µg/day over weeks or months. The IOM also proposed ULs of 25, 38, 63, 75 and 100 µg/day vitamin D for infants and children aged 0-6 months, 6-12 months, 1-3 years, 4-9 years and 9-18 years respectively.

5. In 2012, the dietetic products, nutrition and allergies (NDA) panel of the European Food Safety Authority (EFSA) published a review of vitamin D, which drew on the IOM document (EFSA, 2012). The EFSA panel established a TUL of 100 µg vitamin D per day for adults (an increase from the previous TUL of 50 µg/day (SCF, 2003), and TULs of 25, 50 and 100 µg/day vitamin D for infants and children aged up to 12 months, 1-10 years and 11-17 years respectively. A TUL is intended to apply to all groups of the general population, including more sensitive individuals, throughout life stages such as pregnancy, but with the exception in some cases of discrete, identifiable sub-populations who may be especially vulnerable to one or more adverse effects (e.g. those with unusual genetic predisposition, certain diseases, or receiving the nutrient under medical supervision) (EFSA, 2006).

Method of review

6. The IOM and EFSA documents were used as the initial bibliographic sources for the COT's evaluation. Additional references were identified through an updated literature search using the same terms and searching for publications from 2010 onwards, and from citations in references included in the IOM and EFSA reviews. Where a topic had not been considered by EFSA or IOM, a targeted literature search was conducted using search terms as appropriate. The full search strategy is set out in Annex 1.

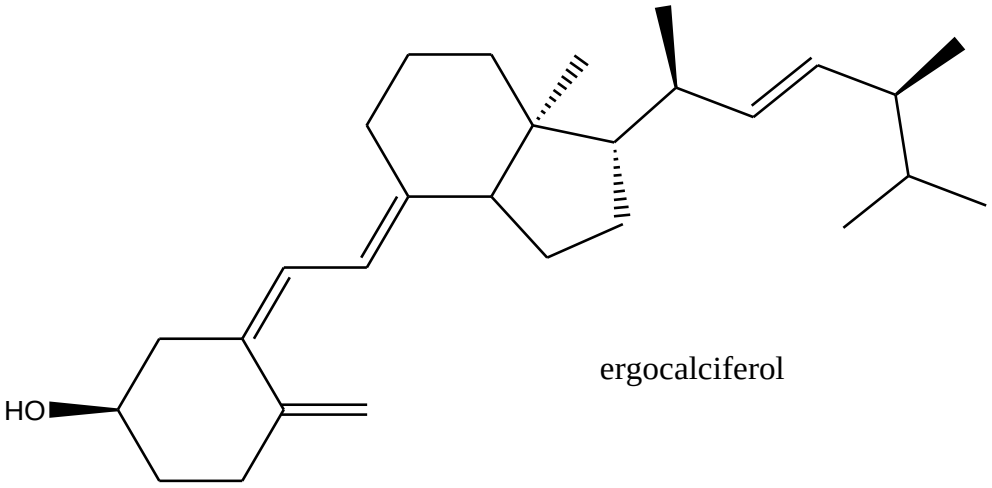
Background

7. Vitamin D is also known as calciferol and comprises a group of fat soluble seco-sterols. The two major forms are vitamin D_2 (ergocalciferol) which is produced by UV irradiation of plant and fungal materials (Lips, 2006; Jäpelt and Jakobsen, 2013) and vitamin D_3 (cholecalciferol) which is synthesised in the skin when it is exposed to UV irradiation. The two forms of vitamin D vary only in their side chains and, while minor differences have been reported, this does not substantially affect their metabolism or biological efficacy (see Fig 1.).

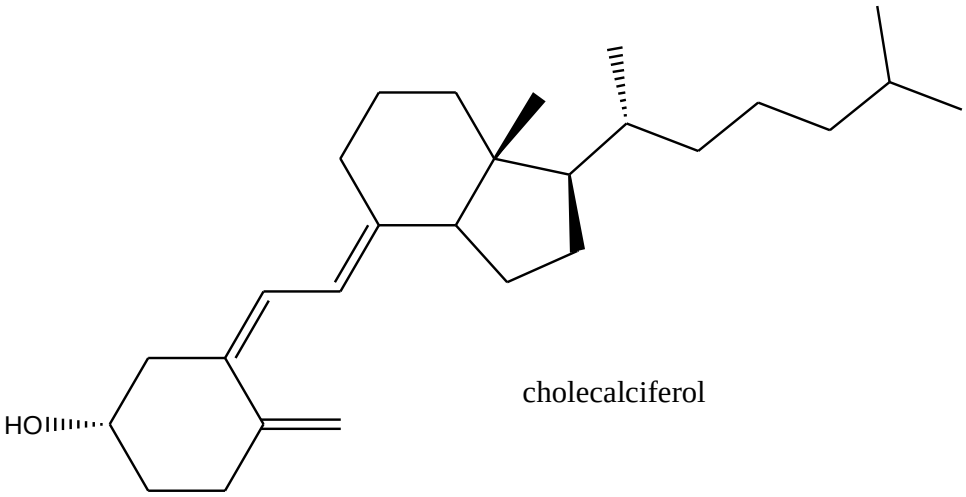
8. Vitamin D requires metabolic transformation before it becomes metabolically active, This transformation entails sequential hydroxylations to form 25

hydroxyvitamin D (25(OH)D) and then 1,25-dihydroxyvitamin D (1,25$(OH)_2$D) also known as calcitriol, which is the active form of vitamin D (IOM,2011).

Fig 1 Structure of vitamin D



ergocalciferol



cholecalciferol

Functions of vitamin D – regulation of calcium and phosphate

9. Vitamin D has a critical role in the metabolism of calcium and phosphate, which are essential for the mineralisation of bone. Its main actions are to increase absorption of calcium from the intestine and to mobilise calcium from bone thereby helping to maintain the normal serum levels of calcium that are required for the

functioning of nerves and muscles. The molecular mechanisms for these processes have not yet been fully elucidated.

10. The regulation of calcium and phosphate involves complex feedback systems. When serum calcium is low, parathyroid hormone (PTH) stimulates reabsorption of calcium in the distal tubules of the kidney, resorption from bone, and also formation of the activating enzyme, 1α-hydroxylase, in the kidney, leading to higher levels of active $1,25(OH)_2D$, As serum calcium rises, PTH secretion falls. In addition, $1,25(OH)_2D$ suppresses parathyroid gene expression and parathyroid cell proliferation by binding to the vitamin D receptor (VDR) in the nuclei of cells in the parathyroid gland, providing additional negative feedback.

11. Production of $1,25(OH)_2D$ is also stimulated by low levels of serum phosphate. The higher levels of serum calcium that then occur as a result of $1,25(OH)_2D$ activity lead to reduced secretion of PTH, which lowers excretion of phosphate by the kidney.

Other possible functions of vitamin D

12. VDRs are distributed widely throughout the body, including in the cells of systems that are not involved in calcium or phosphate homeostasis, such as epidermal keratinocytes, activated T cells of the immune system, antigen-presenting cells, macrophages, monocytes and cytotoxic T cells (IOM, 2011). The presence of VDRs in these cells suggests that vitamin D might have other roles, or that ligands other than calcitriol may also activate VDRs. The tissues concerned also contain 1 α-hydroxylase (cytochrome P450 (CYP) 27B1), which can produce the active form of the vitamin (Bikle, 2009).

13. Vitamin D-responsive elements are present in a large number of genes associated not only with the maintenance of serum levels of calcium and phosphate, and related functions, but also with other possible roles of the vitamin, such as the regulation of cell proliferation, cell differentiation and apoptosis. It has been suggested that calcitriol exerts immunomodulatory and anti-proliferative effects through autocrine and paracrine pathways (Adams and Hewison, 2008).

Vitamin D status

14. The concentration of 25(OH)D in the serum is the best indicator of an individual's longer term vitamin D status, since the active form, $1,25\ (OH)_2D$, has only a short half-life and its formation is modified by factors including PTH (IOM, 2011). Even in severe vitamin D deficiency, levels of $1,25(OH)_2D$ may be normal or elevated through up-regulation of the 1-α hydroxylase enzyme. The circulating level of 25(OH)D in the blood is normally in the range 25-200 nmol/L[3] (Jones, 2009) but Hollis (2005) reported that in sunny environments where cultural practices permit sun exposure, circulating levels are 135 to 225 nmol/L.

15. In the UK, data up to 2012 from the National Diet and Nutrition Survey (NDNS) rolling programme indicated mean 25(OH)D levels of 44.9 and 41.1 nmol/L

[3] Serum 25(OH)D is sometimes reported in ng/ml; 1 ng/ml = 2.49 nmol/L

in boys and girls aged 11-18 years, and 43.5 and 47.3 nmol/L in men and women (Bates, 2014). Levels of 25(OH)D at the 97.5 centile were 100.0 and 87.5 nmol/L in boys and girls aged 11-18 years and 92.4 and 106.0 nmol/L in men and women. The dietary intakes for these groups are summarised in paragraph 21 below.

Sources of vitamin D

UV exposure

16. The major source of vitamin D is its UV-dependent formation in the skin. In the absence of supplementation, most circulating vitamin D_3 is derived from this source.

17. Exposure to summer sun sufficient to generate a minimal reddening of skin (a "minimal erythemic dose" or MED) could generate and release 250-500 µg vitamin D_3 into the circulation within 24 hours (Hollis, 2005). The duration of exposure that is needed to produce this effect will depend on skin pigmentation. For example, a 10-12 minute exposure producing a MED in Caucasians, might require 30 minutes in Asians and 120 minutes in Afro-Caribbean people (Hollis, 2005). Consistent with this, the IOM noted evidence that darker skin was associated with a smaller increase in 25(OH)D for a given dose of UV (IOM, 2011). Latitude, season, use of sunscreen and clothing also affect the formation of vitamin D in the skin.

18. Vitamin D_2 is produced in yeasts and fungi through solar irradiation of ergosterol, and vitamin D_2 in plant material may be a consequence of fungal contamination (Jäpelt and Jakobsen, 2013).

Food and dietary supplements

19. Vitamin D_2 is found in foods of non-animal origin (particularly fungi), and vitamin D_3 in foods such as fatty fish, fish liver oil and egg yolk. In addition, some foods, such as milk, margarine and breakfast cereals, may be fortified with vitamin D. In the UK, the largest contributors to dietary vitamin D intake are meat and meat products, except in children aged 1.5-3 years, among whom the largest contribution comes from milk and milk products. "Fat spreads", "cereals and cereal products" and "buns, cakes, pastries and fruit pies" are also important contributors to vitamin D intake across all age groups (Bates, 2014).

20. Both forms of vitamin D are found in dietary supplements. At present, no maximum levels are prescribed for vitamins and minerals in food supplements, Most multi-vitamin supplements contain 5 µg vitamin D per daily dose, this being the EU Recommended Daily Amount, a harmonised value used for labelling purposes (EC, 2008). However, single supplements are available containing up to 250 µg vitamin D per daily dose, a value in excess of the maximum intakes recently recommended by IOM and EFSA. NHS Healthy Start vitamin drops, which are given free of charge to pregnant women, women with a child under 12 months of age and individuals on low incomes receiving healthy start vouchers, provide 10 µg vitamin D/day for adults and 7.5 µg vitamin D/day for children (NHS, Healthy Start, 2014).

21. In the UK, data up to 2012 from the NDNS rolling programme indicate that mean and high level dietary intakes of vitamin D from food sources (excluding supplements) at ages 11-18 years were 2.4 and 5.7 µg/day in boys, and 1.9 and 4.9 µg/day in girls (Bates, 2014). In men and women aged >18 years, mean and high level intakes were 3.1 and 9.2 µg, and 2.6 and 7.5 µg respectively. In the same groups, mean and high level dietary intakes of vitamin D from combined food and supplement sources were 2.6 and 7.7 µg/day in boys, 2.1 and 6.6 µg/day in girls, 3.9 and 12.3 µg/day in men, and 3.4 and 11.8 µg/day in women.

22. Human milk contains only low levels of vitamin D, but infant formula is fortified with 1-2.5 µg vitamin D/100 Kcal (EC, 2006).

23. The Department of Health currently recommends that most individuals can obtain all the vitamin D they need from "getting some summer sun", and by eating a healthy balanced diet, but that some population groups should take supplements. These groups are: pregnant and breast-feeding women; children aged 6 months to 5 years; infants who are entirely breast-fed or consuming only small quantities of infant formula, and whose mothers have not taken supplements during pregnancy or lactation; and adults aged >65 years who are not exposed to much sunshine (NHS Choices, 2014).

Absorption, distribution, metabolism and excretion

Dermal synthesis

24. The synthesis of vitamin D_3 in the skin is a two stage process. It begins with the irradiation of 7 dehydrocholesterol by UV, which results in the formation of pre-vitamin D (Webb, 2006). Pre-vitamin D then undergoes thermal isomerisation to form vitamin D_3. Irradiation of pre-vitamin D also results in the formation of the inactive compounds lumisterol and tachysterol (Bikle, 2011), and pre-vitamin D_3 can revert back to 7 dehydrocholesterol. The formation of pre-vitamin D_3 is rapid and reaches a maximum within hours after exposure to sunlight. Continued irradiation results in the formation of lumisterol (but not tachysterol or further pre-vitamin D_3). Lumisterol can form pre-vitamin D if levels of the latter fall.

25. Prolonged exposure to sunlight does not lead to toxic levels of vitamin D_3. This is because of the switch to formation of tachysterol and lumisterol, and because vitamin D_3 can be further photoconverted to suprasterols I and II and 5,6 trans-vitamin D_3, all of which are inactive. Prolonged UV irradiation results in a quasi-equilibrium mixture of isomers. The relative amount of each isomer depends on the spectrum and duration of irradiation, but in sunlight there is a limit to the proportion of pre-vitamin D_3 within the mixture, this being less than 12-15% (Webb, 2006). Vitamin D_3 that is formed in the skin enters the circulation attached to vitamin D binding protein (DBP), and is transported to the liver where hydroxylation to 25(OH)D occurs. Subsequent metabolism is the same as for oral vitamin D and is described below. Vitamin D is cleared from the skin within hours.

Oral uptake and distribution

26. Vitamin D is fat soluble and is absorbed in the small intestine with dietary fats. Efficient absorption depends on the presence of fat in the lumen, which triggers the release of bile acids and pancreatic lipase.

27. Within the intestinal wall, most of the ingested vitamin D is incorporated into chylomicrons with cholesterol, triglycerides, lipoproteins and other lipids. The chylomicrons then reach the systemic circulation via the lymphatic system.

28. Chylomicron lipids are metabolised in peripheral tissues that express lipoprotein lipase, particularly adipose tissue and skeletal muscle. The vitamin D that is released may then redistribute onto other carriers such as DBP, albumin and lipoproteins (Haddad *et al.,* 1993), and thereby reach the liver. In addition, after hydrolysis, chylomicron remnants remain. These are cholesterol-rich, triglyceride-depleted particles containing some of the original vitamin D content, and again are transported to the liver.

29. During the hydrolysis of chylomicron triglycerides, a further part of the vitamin D that is present in the chylomicron may be retained locally. In particular, because of its hydrophobic nature, vitamin D is sequestered in adipose tissue. However, its accumulation and mobilisation may simply be passive and it is unclear whether this is subject to any metabolic controls (IOM, 2011).

30. Once vitamin D enters the circulation from the lymph or skin it is cleared within a few hours, by the liver or sequestration.

31. As well as incorporation into chylomicrons, a fraction of ingested vitamin D enters the portal system along with amino acids and carbohydrate, and is transported to the liver directly.

Subsequent metabolism

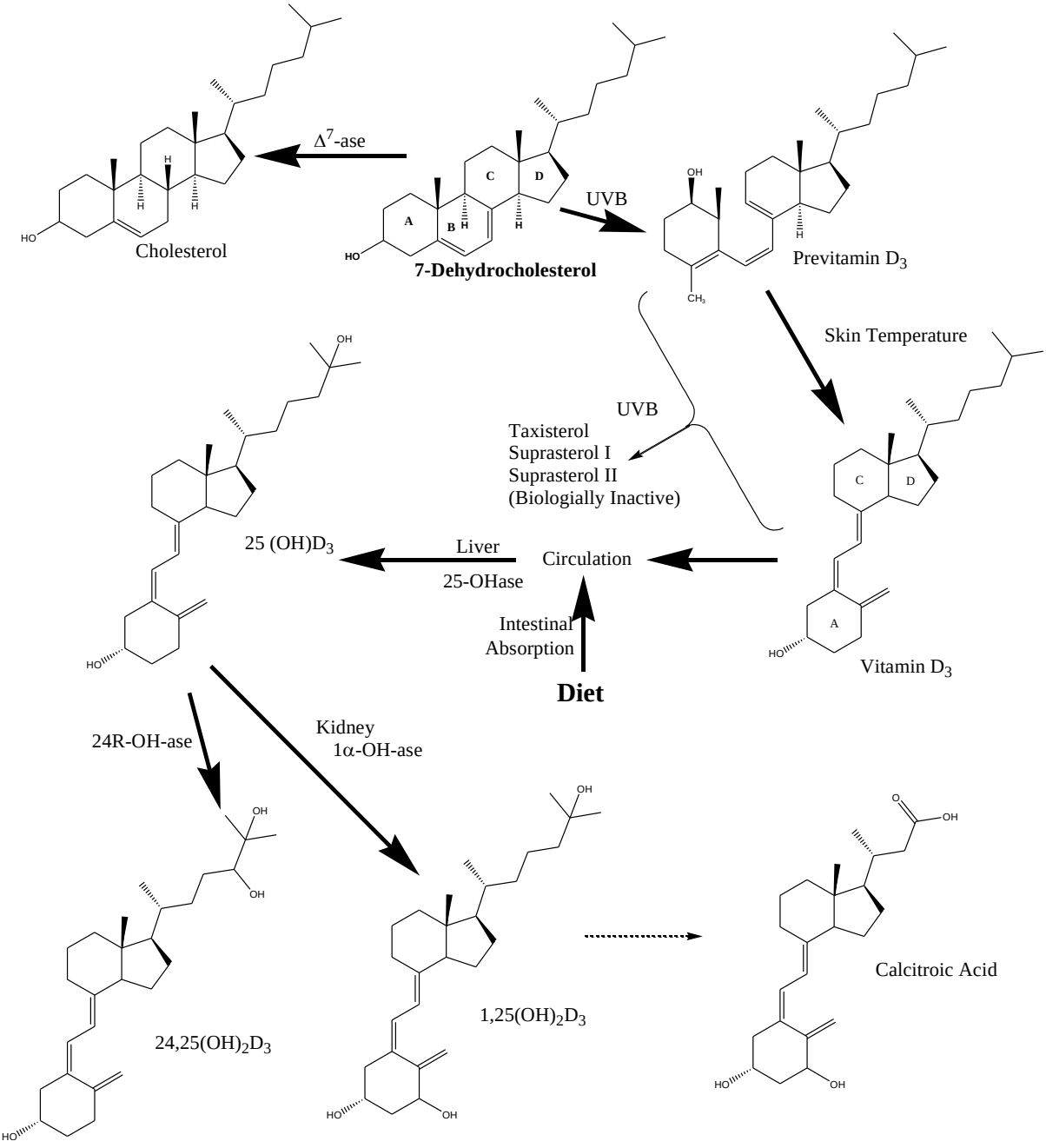
32. Vitamin D is hydroxylated in the liver to 25(OH)D by the 25 hydroxylase enzyme, which is probably CYP2R1 (IOM, 2011), and which appears to be subject to little, if any, feedback regulation. 25(OH)D then circulates in the blood bound to albumin and to DBP (which has a high homology to albumin).

33. In response to changes in PTH stimulated by low serum calcium, a second hydroxylation reaction takes place in the kidney where 25(OH)D is converted by 1α-hydroxylase (CYP27B1) to 1,25$(OH)_2$D. This active metabolite circulates bound to DBP, enters cells and binds to the VDR. The resultant complex then forms a heterodimer with the retinoid receptor and can bind to vitamin D responsive elements on genes such as those for osteocalcin and calcium binding protein. This is followed by transcription and translation of proteins.

34. When 1,25$(OH)_2$D is sufficiently available, calcium levels increase and PTH levels fall, reducing PTH-mediated suppression of another enzyme, 24 hydroxylase (CYP24A1). This results in 1,25 $(OH)_2$D being metabolised to inactive 24,25 dihydroxyvitamin D (24,25$(OH)_2$D) in the kidney, which is then further catabolised by CYP24A1 (Lips, 2006; Jones 2012). The CYP24A1 enzyme is found in many

tissues, including the kidney, and is induced by an interaction of 1,25 $(OH)_2D$ with the VDR. CYP24A1 is also responsible for the metabolic degradation of 25(OH)D and ultimately produces calcitroic acid from calcitriol and 1- desoxycalcitroic acid from 24,25$(OH)_2D$. Vitamin D metabolism is summarised in Fig 2 below:

Fig 2. The metabolism of vitamin D_3 from synthesis/intake to formation of metabolites.



Adapted from IOM, 2011

35. The vitamin D metabolites in the circulation are bound to DBP with a varying degree of affinity, but it is generally higher than, or comparable to, that of 25(OH)D (Jones, 2008). At any one time, only 1–2% of the DBP sterol-binding sites are occupied, and this excess capacity suggests that the primary biological role of DBP might extend beyond acting as a transport molecule for vitamin D (Gomme and Bertolini, 2004). DBP is also involved in binding of fatty acids and sequestration of actin, and may be involved in the modulation of immune and inflammatory responses.

Excretion

36. Vitamin D metabolites are largely excreted through the bile and into the faeces. Very little is eliminated in the urine, partly due to renal reuptake of vitamin D metabolites bound to DBP. The mono, di and tri-hydroxylated metabolites show progressively increasing polarity, culminating with the water-soluble biliary form, calcitroic acid. The whole body half-lives of vitamin D, 25(OH)D and $1,25(OH)_2D$ are 2 months, 15 days and 15 hours respectively (Jones, 2008).

D_2 and D_3

37. Whether there is a difference in potency between the two forms of vitamin D is uncertain. Qualitatively, vitamins D_2 and D_3 exhibit virtually identical biological responses throughout the body, these being mediated by the VDR (IOM, 2011). There is some suggestion from animal studies that vitamin D_2 is less toxic than vitamin D_3, and data from human trials suggest that it is also less effective at increasing serum levels of 25(OH)D. This possibility was discussed in more detail by IOM (2011), who argued that while firm conclusions could not be drawn, at low doses the two forms appeared to be equivalent but at high doses vitamin D_2 appeared less effective, and might also be less toxic. Any such differences could be due to minor differences in metabolism.

38. *In vitro* evidence suggests that rates of inactivation are virtually identical for the two forms of vitamin D. However, the routes of catabolism of $1,25(OH)_2D_2$ and $1,25(OH)_2D_3$ differ following the initial deactivating 24-hydroxylation step. As a result of the actions of non-specific enzyme systems as well as phase II enzymes, the overall half-life of $1,25(OH)_2D_2$ is slightly lower than that of $1,25(OH)_2D_3$. The biliary metabolites of vitamin D_2, are similar to those of vitamin D_3.

39. In this statement, the forms of vitamin D to which observations relate are specified where they have been reported.

Vitamin D toxicity

40. Excessive intakes of vitamin D can cause various adverse health effects, both in humans and in other mammalian species, as a consequence of hypercalcaemia. In addition, some studies have reported adverse effects that may be unrelated to hypercalcaemia.

Effects on calcium metabolism

Hypercalcaemia

41. Despite normal controls on the conversion of 25(OH)D to active $1,25(OH)_2D$, unusually high intakes of vitamin D can lead to hypercalcaemia and hypercalciuria. This occurs through increased absorption of calcium from the gut and resorption of calcium from bone, and can result in deposition of calcium in soft tissues, diffuse demineralisation of bones, and irreversible renal and cardiovascular toxicity. Resorption of calcium from bone may be the most important driver of the hypercalcaemia (Selby *et al*., 1995).

42. Clinical symptoms and signs may include anorexia, nausea, vomiting, weakness, lethargy, constipation and non-specific aches and pains (Barrueto *et al*., 2005), as well as thirst, polyuria, weight loss and cardiac dysrhythmias. These effects have been described in a number of case reports of vitamin D intoxication. Hypercalcaemia has also been reported in a few individuals taking part in trials of vitamin D supplementation.

43. Because of the lipophilicity of vitamin D and its retention in adipose tissue, the effects of vitamin D toxicity can persist for more than two months after the high exposure has ceased (Barrueto *et al*., 2005).

Calcium status and the definition of hypercalcaemia

44. Free (ionised) calcium is the biologically active form of calcium, but calcium is also present in serum bound to protein or complexed with anions. In the blood and extracellular fluid, total calcium is maintained at a concentration of approximately 2.5 mmol/L (range 2.25-2.6 mmol/L) and ionised calcium at 1.1-1.4 mmol/L (EFSA, 2012). Hypercalcaemia is generally defined as a total calcium concentration greater than 2.75 mmol/L. However, the same adverse effects can occur from elevation of ionised calcium even when the total calcium concentration is below this level.

45. Since serum total calcium concentration might not accurately reflect the concentration of biologically active calcium, it is sometimes adjusted for protein or albumin concentration to give a more relevant measure. Most studies in which serum calcium has been measured have determined total or total corrected calcium. The COT noted that while the imperfect correlation between total serum calcium and ionised serum calcium might have implications for the clinical management of individual patients, averaged measurements of total serum calcium should be sufficient for detection of treatment-related changes in populations or groups of individuals.

46. When serum calcium increases above 3 mmol/L, the ability of the kidney to reabsorb calcium is exceeded and hypercalciuria can ensue. Hypercalciuria is defined as being when urinary excretion of calcium exceeds 250 mg/day in women and 275-300 mg/day in men. Urinary calcium should ideally be measured in a 24 hour collection, or be corrected for creatinine to account for dilution. The COT noted that uncorrected concentrations of calcium in spot samples of urine were unlikely to provide useful information, because of variations in the excretion of water.

Case reports

47. As noted above, a number of cases of vitamin D intoxication have been reported in the literature. These cases, which are summarised in Table 1 below, did not occur through normal dietary exposure, but resulted from high medicinal doses or excessive use of supplements, often over a sustained period.

48. Cases of intoxication have generally been associated with serum 25(OH)D levels of at least 300 nmol/L, and often exceeding 1000 nmol/L. Where lower serum levels of 25(OH)D have been reported, this may have been because the blood sample was not collected soon after the over-exposure occurred.

Table 1: Case reports of vitamin D intoxication.

Population	**Dose/ Exposure µg**	**Duration**	**Serum 25(OH)D nmol/L**	**Serum/plasma Ca mmol/L**	**Symptoms**	**Reference**
Boy 2y	Liquid supplement. 15,000 D_2/day	4 days	1175 (peak)	3.6	Colic constipation, vomiting lethargy	Barrueto *et al*., 2005
Infant 3 mo Infant 4 mo	750/day 1500/day	45 days 1 month		4.4 4.9	Lethargy, dry oral mucosae Lethargy, vomiting, failure to thrive	Beşbaş *et al*., 1989
Boy 6 y	1250/day "calciferol" tablets	9 months			Thirst, bed wetting (diabetes insipidus)	Dent, 1964.
Boy 5 ½ y	2500 vitamin D + cod liver oil and multivitamins	2-3 months for all, vitamin D carried on for 1 y afterward.		4.25	Irritability, restlessness, nausea, lumps on tibia. Patterns of increased and decreased bone density in X rays. Loss of bone density and tissue calcification persisted after vitamin D stopped. Osteosclerosis, severe calcinosis, fatal renal failure.	De Wind., 1961
Female 66y	5 (2 x day). Symptoms started with new pack 200 µg/pill	3 years	696	4.04	"severe constitutional symptoms" anaemia	Puig *et al*., 1998
Male 32y	Lab technician working with vitamin D_3 dust	65 days in a 3 y period	1240 (1 month post exposure)	3.5	Polydipsia, anorexia nausea, general malaise	Jibani and Hodges, 1985
Female 58y	Supplements containing 4674 D_3 per serving	2 months	1171	3.75	Fatigue, forgetfulness, constipation, back pain, nausea, vomiting.	Klontz and Acheson, 2007
Male 42y	Supplement powder – variable D_3 content. 3900-65100/day consumed	2 years	1218	3.75	Hypercalcaemia	Koutkia *et al*., 2001
Female 70 y	15000 D_3/day	3 weeks	1474	3.05	Hypercalcaemia not apparent when 25(OH)D declined to 711 nmol/L 9 wks after vitamin D withdrawn.	Lilienfeld-Toal *et al*., 1978
Female 49 y	2500 D_3	6 years	706, 666		Weight loss, anorexia, pruritis, back pain and bone pain. Hypercalcaemia	Streck *et al*, 1979
Male 69y	2500	3 weeks		5	Abnormal ECG. Patient also had a	Sterling and Rupp,

Population	Dose/ Exposure µg	Duration	Serum 25(OH)D nmol/L	Serum/plasma Ca mmol/L	Symptoms	Reference
					tumour which may have contributed to the hypercalcaemia.	1967
Female 70 y	1250/day	3 months			Confusion, unstable gait, slurred speech, fatigue.	Jacobsen *et al*., 2011
Male 58 y Male 40 y	46,600 D_3 24,300 D_3	2 months 1 month	3045 1610	3.75 3.3	Fatigue, thirst, polyuria, poor mental activity. Nausea, vomiting, thirst, polyuria, muscle aches.	Araki *et al*., 2011
7 cases (3 adults, 4 children)	Unknown source of D_3	Unknown (1 week for 2 adults)	832-1287	2.72-4.08	Vomiting, anorexia, constipation, polydipsia	Thomson and Johnson, 1986
Family, 2 adults + 11 month infant	Food cooked in nut oil – Vit D_3 125000 µg /ml	Not stated, (IOM say single exposure)	600- 3750	4-4.3	Nausea, vomiting, abdominal pain, weakness and sensory loss. Suffused conjunctivae. Negative calcium balance suggesting Ca mobilisation from bone. Miscarriage at 10 weeks. 11 years later, all well but persistent nephrocalcinosis in adult male.	Down *et al.,* 1979
8 patients (7 aged 39-82y, 1 aged 15 mo)	Milk – 118-710 ml/day consumed. D_3 levels "not detected" to 5814 µg /L)	Sporadic excess in milk.	Mean = 293± 435	3.15 ± 0.5	Weight loss, anorexia, fatigue, weakness, vomiting, constipation	Jacobus *et al*., 1992
2 children 3 mo 7 mo	 - 300		 321 314	"Elevated"	Anorexia, diarrhoea, vomiting (IOM) Hypercalcaemia	Jacqz *et al*., 1985 Abstract only
4 infants 14 mo 8 mo 10 mo 10 mo	 250-750 D_2/day 250-750 D_2/day 600-1000 D_2/day 600-1000 D_2/day	 12 mo 5 mo 8 mo 8 mo		 4.45 4.68 - -	Fever, anorexia, weight loss, skeletal changes. Latter 2 cases fatal.	Ross, 1952
Males, 63 and 29y	Approximately 1.3 g/ month in table sugar	7 months	3700 and 1555	4.39 and 3.82	Right sides flank pain. Conjunctivitis, anorexia, fever, chills, weight loss, thirst, vomiting	Vieth *et al*., 2002

Population	**Dose/ Exposure µg**	**Duration**	**Serum 25(OH)D nmol/L**	**Serum/plasma Ca mmol/L**	**Symptoms**	**Reference**
11 patients, 8-69 y	Vitamin D concentrate 50,000 µg /g used as cooking oil	Single exposure	847-1652	Mean = 3.99± 0.3	Abdominal cramps, vomiting, neurological symptoms	Pettifor *et al*., 1995
6 cases (14-57y) –variety of indications UK	2500 D_2/day 5000 D_2/day 2500 D_2/day 2500 D_2/day ? 5000 D_2/day	10y 2 y 10y 13 y ? 2y	866 802 1005 533 643 1203 Mean = 842	3.19 3.00 3.24 3.31 3.07 3.77 Mean = 3.22		Selby *et al*., 1995
7 cases (50-84y) Treated with vitamin D for osteo-porosis, osteomalacia or hypopara-thyroidism	250-750 D_3	Various- 3 wks to 7.2y	710 ± 179 mean (range 221-1692)	Mean = 3.3 ± 0.25	Asthenia, weight loss, nausea, polydipsia, polyuria, bradypsychism, sleepiness, pruritus, dizziness, episcleritis Urinary calcium 0.192 ± 0.067 mmol/L	Rizzoli *et al*., 1994
4 cases (all female) 42-77y Patients with osteoporosis or osteomalacia	1250 µg /? 1250 D_2µg/week 1250 µg /day 1250 D_2µg /2 x week	>14 months 6 weeks 6 weeks 5 years	354 - 319 586	3.35 2.88 3.75 -	Lethargy, tenderness in some joints, pain, nausea, weakness, confusion, hypertension.	Schwartzman and Franck, 1987
8 cases (15-60y) Female 71y (only case with 25(OH)D	1250 – 5000 D_2 /day 3750 D_2/day	4 months-10y 2y	 1123	3.1-4.4	Back ache, sore eyes, nausea, vomiting, anorexia, pruritus, polydipsia. Polyuria. No symptoms in 1 patient.	Davies and Adams, 1978

Population	Dose/ Exposure µg	Duration	Serum 25(OH)D nmol/L	Serum/plasma Ca mmol/L	Symptoms	Reference
10 cases (48-75y)	525,000 270,000 ? ? 300,000 ? 1,350,000 75000 ? 1,500,000	1-4 months	302 172 200 165 164 283 100 236 176 306	3.38 3.0 3.48 3.1 3.2 3.1 3.5 3.4 3.2 3.55	Lassitude, vomiting, polyuria, polydipsia, altered sensorium, anorexia, oliguria. NB. The patients were from an area of India with endemic hypovitaminosis D.	Koul *et al*., 2012

Hypercalcaemia in intervention studies

49. For the purposes of risk assessment, the information provided by case reports of vitamin D intoxication is limited insofar as exposures have varied in level and duration, and have not always been well characterised. Trials of vitamin D supplementation provide better documentation of dosage.

50. Numerous supplementation studies have been conducted in human volunteers with the aim of improving vitamin D status and/or measures of bone health. Often they have focused on population groups such as the elderly, who are more liable to low vitamin D status and its consequences.

51. The relevant studies (identified as being capable of detecting adverse effects of vitamin D supplementation) have been tabulated in Annex 2 which provides information about the populations studied and dosing regimens, as well as on serum calcium and 25(OH)D concentrations and reported adverse effects. The studies have been grouped into randomised controlled trials (RCTs) that were included in a systematic review commissioned as part of the IOM review (Appendix C to IOM, 2011), other studies cited by IOM, and additional studies identified by EFSA. No other relevant studies were found in the updated literature search. Trials varied widely in design, though there were few that employed daily doses of vitamin D greater than 100 µg, and where higher daily doses were used, it was rarely for longer than a few months. Only two studies (Heaney *et al*., 2003 and Barger-Lux *et al*., 1998) used one or more dose levels ≥ 100 µg vitamin D/day in the absence of calcium supplements, for ≥2 months.

52. Only isolated instances of hypercalcaemia were reported in these supplementation studies. In some trials mean calcium levels increased, but remained within the normal range.

53. The two key studies by Heaney *et al*., 2003 and Barger-Lux *et al*., 1998 are described further in paragraphs 85-86 in the section which concerns the establishment of a TUL.

Kidney stones

54. Prolonged hypercalciuria is a risk factor for kidney stones, and while available human studies suggest that high intakes of vitamin D alone are not associated with an increased risk of kidney stones, there may be a hazard from combined supplementation with calcium. Jackson *et al*., (2006) reported an increased risk of kidney stones in women given a daily supplement of 1,100 mg calcium plus 10 µg vitamin D for up to seven years (taking into account the diet, estimated total daily intakes were 2,100 mg calcium and 20 µg vitamin D). However, the total intake of vitamin D in this study was not at a level associated with hypercalcaemia.

Other possible adverse effects

Falls and fractures

55. In an intervention study by Sanders *et al*., (2010), 2,256 community-dwelling Australian women who were aged ≥ 70 years and considered to be at a high risk of fracture, were randomly assigned to receive single doses of 12,500 µg vitamin D_3 or placebo annually for 3-5 years. Women in the treatment group experienced 171 fractures compared to 135 in the controls; and 837 women in the treatment group fell 2,892 times (rate 83.4 per 100 person years) compared to 769 women in the placebo group who fell 2,512 times (rate 72.7 per 100 person years). The incidence rate ratio (IRR) for fracture was 1.15 (95%CI 1.02-1.30), and that for falls 1.26 (95%CI 1.00-1.59). Furthermore, a temporal relationship to treatment was observed in a *post hoc* analysis. The IRR for falling in the vitamin D group was 1.31 (95%CI 1.12-1.54) in the first 3 months after dosing and 1.13 (95%CI 0.99-1.29) at other times. A similar pattern was observed for fractures but the difference in risk was not statistically significant. There were no differences in the incidence of other serious adverse events between the treatment and control groups.

56. In a sub-study of Sanders *et al*., (2010), 137 participants (75 from the vitamin D group and 58 from the placebo group) underwent serial blood sampling for 25(OH)D and PTH levels. At baseline, the median 25(OH)D concentration was 49 nmol/L with fewer than 3% of participants having levels lower than 25 nmol/L. In the vitamin D group, 25(OH)D levels increased to 120 nmol/L one month after dosing, were approximately 90 nmol/L at three months, and remained higher than those in the placebo group 12 months after dosing. Data on serum levels of calcium were not reported.

57. The authors noted several limitations of the study. In particular, it had not been possible to measure biochemical parameters in all of the participants. However they considered that subjects would not have reached a “toxic” 25(OH)D concentration of 375 to 400 nmol/L because in the sub-study, the highest level measured one month after dosing was 208 nmol/L. Since 25(OH)D levels were thought to peak 7-21 days post-treatment and decrease slowly thereafter, it was considered likely that peak levels would be only marginally higher than the one-month values. The authors also commented that increases in serum concentrations of 25(OH)D were likely to be lower in individuals who were replete before supplementation.

58. The study by Sanders *et al*., (2010) followed on from an earlier trial by Trivedi *et al*., (2003), in which oral doses of 2500 µg vitamin D or placebo were given every four months for five years to 2686 community-dwelling men and women (1345 treatment, 1341 controls) aged 65-85 years, who were living in England. In the Trivedi *et al*., (2003), study the risk of fracture in the treated group was reduced compared to the placebo group (RR 0.78, 95%CI 0.48-0.93), and there were no documented adverse effects.

59. In contrast, a study by Smith *et al*., 2007, found an increase in non-vertebral fracture in women, although not in men, who were given an annual intra-muscular injection of 7500 µg vitamin D_2. No effect was observed on the frequency of falls, which was a secondary outcome assessed by six-monthly recall. Cauley *et al*., 2011 reported that in a case control study nested within the Women’s Health Initiative in the USA, higher 25(OH)D levels were associated with a lower risk of fracture in white

women. On the other hand, 25 (OH)D levels ≥ 50 nmol/L were associated with an increased risk of fracture in black women (OR 1.45, 95%CI 1.06-1.98) and 25(OH)D levels ≥ 75 nmol/L were associated with a higher risk of fracture in Asian women, but only after adjustment for DBP (OR 2.78, 95%CI 0.99-7.88). There was no association in Hispanic or Native American women.

Pancreatic cancer

60. Several observational studies have suggested an association between higher vitamin D intakes or serum 25(OH)D levels and the risk of pancreatic cancer, but the finding has not been universal. Skinner *et al*., 2006 and Giovannucci *et al*., 2006a did not find such an association, whereas it was observed by Stolzenberg-Solomon *et al*., 2006, 2009 and 2010.

61. Skinner *et al*., (2006) reported data from two large prospectively followed cohorts; 46,771 men aged 40-75 years in the Health Professionals Follow-up Study (HPFS) and 75,427 women aged 38-65 years in the Nurses' Health study. In these studies, 365 incident cases of pancreatic cancer were identified over 16 years of follow-up. Analysis indicated that higher vitamin D intakes at baseline were associated with a lower risk of pancreatic cancer. Compared with participants in the lowest category of vitamin D intake (<3.75 µg/day) pooled multivariate risk estimates were as shown in Table 2 below.

Table 2: Relative Risks of pancreatic cancer according to vitamin D intake from Skinner *et al*., 2006

Vitamin D µg/day	**RR (95%CI**)
<3.75	1.0
3.74-7.49	0.78 (0.59-1.01)
7.5- 11.23	0.57 (0.40-0.83)
11.25- 14.99	0.56 (0.36-0.87)
≥ 15	0.59 (0.40-0.88)
P trend = 0.01	

Risk estimates were adjusted for age, time period, total energy intake, smoking, diabetes, BMI, height, region of residence, parity and use of multi-vitamin supplements

It was noted that 95% of men and 94% of women in the highest categories of vitamin D intake used supplements. When these were excluded from the analysis, the inverse relationship between vitamin D intake and pancreatic cancer risk remained.

62. Similarly, in another study of 43,949 men from the HPFS cohort, Giovannucci *et al*, (2006b) reported reduced mortality from all cancers and from digestive cancer (which included pancreatic cancer) with higher predicted serum levels of 25(OH)D[4].

63. In contrast, Stolzenberg-Solomon *et al*., (2006) found a positive association between serum 25(OH)D levels and risk of pancreatic cancer in a case-control

[4] The level of 25(OH)D was predicted from vitamin D intake assessed by food frequency questionnaire and a pilot study measuring 25(OH)D levels in 1095 men from the study.

investigation that was nested within the Alpha-Tocopherol, Beta Carotene Cancer Prevention (ATBC) study cohort of Finnish male smokers. A total of 200 incident cases were compared with 400 controls, and variables examined as potential confounders were age, smoking history, education, residence in city, height, weight, BMI, blood pressure, a range of medical conditions including pancreatitis, dietary nutrient intakes from food, and supplements, alcohol intake, serum nutrients, occupational and leisure-time physical activity, and season. Higher vitamin D concentrations were associated with a 3-fold increase in risk of pancreatic cancer (highest vs lowest quintile, >65.5 vs. <32.0 nmol/L, OR 2.92; 95%CI 1.56-5.48, P_{trend} = 0.001 across the quintiles, that remained after excluding cases diagnosed early during follow-up).

Table 3: Odds Ratios of pancreatic cancer according to levels of serum 25(OH)D (Stolzenberg-Solomon *et al*., 2006)

Serum 25(OH)D nmol/L	**OR (95%CI**)
<32	1.0
>32 and <41.1	1.30 (0.70-2.40)
>41.1 and <51.1	2.12 (1.15-3.90)
>51.1 and <65.5	1.50 (0.81-2.76)
>65.5	2.92 (1.56-5.48)
P trend = 0.001	

Factors adjusted were age, month of blood draw, smoking history, occupational physical activity, education and serum retinol.

64. The same group also conducted a nested case-control study based on the US Prostate, Lung, Colorectal and Ovarian Screening Trial (PLCO) cohort of 152,810 men and women aged 55-74 years at baseline (Stolzenberg-Solomon, 2009). During the follow-up period (11.7 years) 184 incident cases of pancreatic cancer were identified and these were matched with 368 controls. Blood samples and dietary information (including about use of supplements) were obtained at baseline. Odds ratios were estimated by conditional logistic regression, with adjustment for smoking and BMI. Vitamin D concentrations were non-significantly associated with pancreatic cancer (highest vs lowest quintile, >82.3 vs. <45.9 nmol/L, OR 1.45; 95%CI 0.66-3.15, P_{trend} = 0.49).

Table 4: Odds Ratios of pancreatic cancer according to levels of serum 25(OH)D (Stolzenberg-Solomon *et al*., 2009)

Serum 25(OH)D nmol/L	**OR (95%CI**)
≤45.9	1.0
>45.9 and ≤60.3	0.97 (0.47-1.98)
>60.3 and ≤69.5	0.86 (0.40-1.84)
>69.5 and ≤82.3	0.84 (0.-39-1.80)
>82.3	1.45 (0.66-3.15)
P trend = 0.49	

Factors adjusted were age, month of blood draw, smoking and BMI.

65. In an attempt to resolve these inconsistencies, a pooled nested case-control study was conducted as part of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers (1974-2006), using data from several cohorts (Stolzenberg-Solomon *et al*., 2010). In total, 952 incident cases of pancreatic cancer were identified during a median follow up period of 6.5 years and matched to 1,333 controls by cohort, age, sex, race/ethnicity, date of blood draw and follow up time. Conditional logistic regression was used to estimate odds ratios and 95% confidence intervals for pancreatic cancer, adjusted for smoking, BMI and diabetes. No significant elevation of risk was observed with lower levels of 25(OH)D. However, high 25(OH)D concentrations (≥ 100 nmol/L) were associated with a statistically significant two-fold increase in risk of pancreatic cancer of >100 nmol/L compared to those with serum 25(OH)D of 50-<75 nmol/L (OR 2.12, 95%CI 1.23-3.64). The ORs for each sextile are given in Table 5 below.

Table 5: Odds ratios of pancreatic cancer by sextile of serum 25(OH)D from Stolzenberg-Solomon *et al*., 2010

25(OH)D nmol/L	**OR (95%CI)**
<25	1.0
25-37.5	1.04 (0.74-1.44)
37.5-50	1.10 (0.79-1.55)
50-75	1.06 (0.76-1.48)
75-100	1.08 (0.73-1.59)
≥ 100	2.24 (1.22-4.12)
P trend 0.14	

Factors adjusted were age, month of blood draw, smoking history, occupational physical activity, education and serum retinol.

The study cohorts were the ATBC study, Campaign against Cancer and Stroke (CLUE), the Cancer Prevention Study II Nutrition Cohort, the New York University Women's Health Study, the Multi-ethnic Cohort Study, the PLCO study, and the Shanghai Women's and Men's Health Studies. Median serum 25(OH)D levels were comparable between cases and controls, but ranged from 33.4 to 64.7 nmol/L in the different cohorts. The increased risk in the highest category of serum 25(OH)D persisted even after the exclusion of cases diagnosed within the first 2 years after blood samples were collected, leaving 558 cases and 840 controls (OR 2.20, 95%CI 1.22-3.96). Odds ratios were similar when the analyses were restricted to US cohorts only, and when each cohort was excluded in turn. There was no significant interaction with use of vitamin D supplements or multivitamins. Among non-users of supplements the ORs were 0.83, 1.12, 1.10, 1.01 and 4.19 (95% CI 1.73-10.16) compared to the reference group.

66. The findings of Stolzenberg-Solomon were disputed by Baggerly and Garland (2012), who argued that the positive association was a statistical artefact arising from the choice of cut-points and that merging the top two groups largely abolished the relationship.

Prostate cancer

67. Tuoihimaa *et al*., (2004) conducted a nested case-control study of prostate cancer, using stored serum from three cohorts of Nordic men. Serum 25(OH)D levels in 622 cases of prostate cancer were compared with those in 1451 controls matched by cohort. The cohorts included in the study were from the Helsinki Heart Project, the Janus project (Norway) and the Northern Sweden Health and Disease study. It was reported that both low (≤ 19 nmol/L) and high (≥ 80 nmol/L) serum levels of 25(OH)D were associated with higher risk of prostate cancer, while the mid-range concentrations of 40-60 nmol/L were associated with the lowest risk. The ORs from the combined dataset are shown in Table 6 below. Separate analyses by country showed a similar pattern, although the U-shaped relationship was less apparent in the Norwegian cohort and more marked in that from Finland.

Table 6: Odds ratios of prostate cancer according to serum 25(OH)D from Tuoihimaa *et al*., 2004

	All Countries	
25(OH)D level nmol/L	**Number of cases**	**OR (95%CI)**
≤ 19	19	1.5 (0.8-2.7)
20-39	69	1.3 (0.98 -1.7)
40-59	229	1 referent
60-79	138	1.2 (0.9-1.5)
≥ 80	67	1.7 (1.1-2.4)

68. In contrast, Faupel-Badger *et al*., (2007) reported that, in a nested case-control study of men from the ATBC trial of Finnish smokers, there was no association between serum 25(OH)D and risk of prostate cancer. A total of 296 cases of prostate cancer were identified from a cohort of 29,133 men, and compared with 297 controls, conditional logistic regression being used to estimate ORs according to baseline serum 25(OH)D levels (Table 7).

Table 7: Odds ratios of prostate cancer according to serum 25(OH)D from Faupel-Badger *et al*., 2007

	Quartile 1	**Quartile 2**	**Quartile 3**	**Quartile 4**	P_{trend}
Cases/controls	83/75	69/73	57/74	87/75	
25(OH)D nmol/L	≤ 36.75	37- 47.05	47-59.8	>59.8	
Unadjusted OR (95%CI)	1.00	0.81 (0.45-1.47)	0.57 (0.30-1.06)	0.87 (0.49-1.57)	0.99
Adjusted OR* (95%CI)	1.00	0.88 (0.48-1.61)	0.59 (0.31-1.11)	0.89 (0.49-1.62)	0.97

* Factors adjusted were age at randomisation, BMI and pack years of smoking

It was noted that the vitamin D status of the population was skewed towards the low end of the usual range, with more than 25% being deficient. Although the authors considered the results to weigh against an association of vitamin D with prostate cancer, it should be noted that the lowest risk was in the third rather than the highest quartile of serum 25(OH)D.

All-cause mortality

69. Five cohort studies were identified by IOM (2011) that had examined the association between serum 25(OH)D levels and all-cause mortality. In general these indicated that levels <30 nmol/L were associated with an increased risk of death, and that mortality then decreased as serum 25(OH)D increased. However, three of the studies suggested a "U" or "reverse J"-shaped dose-response relationship, with a slight increase in all-cause mortality at the highest serum 25(OH)D levels.

70. In a sample of community-dwelling men (n= 208) and women (n=191) aged older than 75 years in Scotland, Jia *et al*., (2007) observed a statistically significant trend of lower all-cause mortality with increasing serum 25(OH)D levels (*p*= 0.03). Blood samples were taken at baseline, and the median follow-up was 69.2 months. Hazard Ratios (HRs) were estimated by Cox regression. Although it was not discussed by the authors, the IOM considered that a U- or reverse J-shaped relationship was apparent, with the lowest mortality at serum levels of 25(OH)D below 50 nmol/L, and an apparent increase in the highest quintile (47.1-82 nmol/L in men and 39.1- 82 nmol/L in women) compared to the fourth quintile (37.0– 47 nmol/L in men and 30.3-39 nmol/L in women). The HRs for the different quintiles are shown in Table 8 below:

Table 8: Hazard Ratios for all-cause mortality by quintile of serum 25(OH)D from Jia *et al*., (2007)

Quintile	**HR(95%CI)***	**HR(95%CI)‡**	**HR(95%CI)†**	**HR(95%CI)§**
25(OH)D nmol/L				
6-23 (men) 7-19 (women)	2.22 (1.22-4.06)	2.02 (1.10-3.72)	1.97 (1.03-3.75)	1.74 (0.91-3.34)
23.1-30.0 (men) 19.1-24.0 (women)	1.75 (0.95-3.22)	1.64 (0.89-3.02)	1.54 (0.80-2.97)	1.40 (0.73-2.70)
30.1-37.0 (men) 24.1-30.2 (women)	1.03 (0.53-2.00)	1.01 (0.52-1.96)	0.95 (0.48-1.88)	0.90 (0.45-1.79)
37.1-47.0 (men) 30.3-39.0 (women)	0.92 (0.46-1.84)	0.87 (0.43-1.75)	0.86 (0.43-1.74)	0.80 (0.39-1.62)
47.1- 82.0 (men) 39.1-82.0 (women)	1	1	1	1
P for trend	0.001	0.008	0.01	0.03

* adjusted for age and sex, taking ≥5 medicines, self-perceived health status, pre-existing heart disease and/or diabetes, ‡ as * but also adjusted for sunlight exposure, †as * but also adjusted for use of supplements containing vitamin D, §as * but also adjusted for sunlight exposure and use of supplements containing vitamin D.

71. A similar pattern was reported by Visser *et al*., (2006) in a sample of 1260 independent community-dwelling persons aged ≥ 65 years who were participating in the Longitudinal Aging Study Amsterdam. The time between blood sampling and the end of the study was 5-6 years. Mortality was reduced at higher than deficiency levels of 25(OH)D (≥ 25 nmol/L), but was slightly increased for the highest quartile of blood levels (≥75 nmol/L) compared to the third quartile (50-74.9 nmol/L). The HRs are given in Table 9 below:

Table 9: Hazard Ratios for all-cause mortality by quartile of serum 25(OH)D from Visser *et al*., (2004)

Quartile	**Model 1***	**Model 2§**	**Model 3†**	**Model 4‡**
25 OHD nmol/L	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
<25	1.61 (1.09, 2.37)	1.54 (1.04, 2.29)	1.47 (0.99, 2.19)	1.28 (0.85, 1.92)
25-49.9	1.17 (0.85, 1.62)	1.11 (0.80, 1.54)	1.08 (0.78, 1.51)	1.00 (0.72,1.40)
50-74.9	0.93 (0.67, 1.29)	0.95 (0.68, 1.32)	0.95 (0.68, 1.32)	0.91 (0.65, 1.26)
≥ 75	1.0	1.0	1.0	1.0
P for trend	0.0058	0.021	0.046	0.19

* Adjusted for gender, age, and education; **§**as * but also adjusted for chronic disease, serum creatinine concentration, cognitive status, and depressive symptoms; **†**as **§** but also adjusted for lifestyle variables including BMI, smoking status, alcohol consumption, and physical activity; as **†** but also adjusted for frailty indicators: mobility performance, low serum albumin concentration, and low serum total cholesterol concentration.

72. Similar findings were reported by Melamed *et al*., (2008) who linked data from the NHANES III study to mortality outcomes for 13,331 adults aged ≥ 20 years in the USA. Serum 25(OH)D levels were measured at baseline (1988-1994), and the participants were then followed for mortality over a median of 8.7 years. In multivariable analysis, the lowest quartile of serum 25(OH)D was associated with a 26% increase in all-cause mortality when compared to the highest quartile (Table 10).

Table 10: Hazard Ratios for all-cause mortality according to quartile of serum 25(OH)D from Melamed *et al*., (2008)

	Unadjusted	**Limited adjustment***	**Fully adjusted‡**	**Fully adjusted (without diabetes or hypertension)**
25(OH)D nmol/L	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
<44.5	1.78 (1.44-2.21)	1.52 (1.31, 1.77)	1.26 (1.08, 1.46)	1.28 (1.11, 1.48)
44.5-61	1.49 (1.24, 1.78)	1.11 (0.95, 1.31)	1.06 (0.89, 1.24)	1.06 (0.9,1.26)
61-80.1	1.14 (0.94-1.39)	0.92 (0.78, 1.08)	0.93 (0.79, 1.10)	0.94 (0.80, 1.12)
≥ 80.1	1.0	1.0	1.0	1.0

*Adjusted for age, sex, race, season,‡ Fully Adjusted Model included age, sex, race, season, hypertension, history of prior cardiovascular disease, diabetes, smoking, HDL cholesterol, total cholesterol, use of cholesterol medications, estimated glomerular filtration rate (eGFR) categories, serum albumin, log (albumin-creatinine ratio), log C-reactive protein (CRP), BMI, physical activity level, vitamin D supplementation and low socioeconomic status.

In a sub-group analysis, the association between serum 25(OH)D levels and all-cause mortality differed by sex, being more pronounced in women (Table 11):

Table 11: Hazard Ratios for all-cause mortality in men and women by quartile of serum 25(OH)D from Melamed *et al*., (2008)

	Men	**Women**

25 OHD nmol/L	HR (95%CI)	HR (95%CI)
<44.5	1.04 (0.83-1.30)	1.51 (1.15-1.98)
44.5-61	0.94 (0.75,-1.19)	1.27 (0.97,-1.66)
61-80.1	0.82 (0.64-1.05)	1.16 (0.87,-1.55)
≥ 80.1	1.0	1.0
P interaction 0.06		

Adjusted for age, race, season, hypertension, history of prior cardiovascular disease, diabetes, smoking, HDL cholesterol, total cholesterol, use of cholesterol medications, eGFR categories, serum albumin, log(albumin-creatinine ratio), log(CRP), BMI, physical activity level, vitamin D supplementation and low socioeconomic status.

73. In a study of 842 Australian nursing home residents (Sambrook *et al*., 2004), higher serum 25(OH)D levels at baseline were associated with lower all-cause mortality (HR, 0.99; 95%CI 0.98-1.0). However, in analyses which corrected for health status, nutritional status and renal function as well as age and sex, there was no longer a significant relationship. Mean 25(OH)D levels by survivor status were 27.9 nmol/L in subjects who were still alive at the end of follow-up, and 24.9 nmol/L in those who had died. (p =0.006).

74. Semba *et al*., 2009 reported that low serum 25(OH)D levels were associated with increased mortality in a study of 714 community-dwelling women in the USA, aged 70-79 years. During the 7-year follow up period, 100 of the women died. In a multi-variate Cox proportional hazards model that adjusted for demographic variables, season, use of supplements, and other potential confounders, women in the lowest quartile of 25(OH)D concentrations (<38.25 nmol/L) were at higher risk of mortality than women in the highest quartile (>67.5 nmol/L) (HR 2.45, 95%CI 1.12-5.36).

75. Several further studies of serum 25(OH)D levels and all-cause mortality were found in addition to those reviewed by IOM.

76. In a prospective study using data from NHANES III (1988-1994), Ginde *et al*., (2009) investigated all-cause mortality in 3408 adults aged ≥ 65 years over a median follow-up period of 7.3 years. A total of 1493 deaths occurred during this time. A fully adjusted analysis suggested an inverse relationship between all-cause mortality and serum 25(OH)D levels, with no indication of any increase at the very highest levels (Table 12)

Table 12: Hazard Ratios for all-cause mortality by quintile of serum 25(OH)D from Ginde *et al*., (2009)

Serum 25 OHD nmol/L	HR (95% CI)
< 25	1.83 (1.14-2.94)
25-49.9	1.47 (1.09-1.97)
50-74.9	1.21 (0.92-1.59)
75- 99.9	1.15 (0.86-1.53)
100	1.0 (ref)

* Adjusted for age, sex, race/ethnicity, poverty: income ratio, region, BMI, physical activity, smoking, asthma, Chronic Obstructive Pulmonary Disease (COPD), renal function, hypertension, diabetes, hyperlipidaemia, history of MI, stroke and cancer.

77. A further investigation was conducted by Ford *et al*., (2011) using data from the NHANES mortality study from 2002-2004, with a mean follow-up of 3.8 years. Among the 7531 participants aged ≥ 20 y, there were 347 deaths. The mean unadjusted serum concentrations of 25(OH)D at baseline were 54.1 nmol/L in the participants who subsequently died, and 60.7 nmol/L in the survivors (p= 0.002). After adjustment for socio-demographic factors, the hazard ratio was 1.65 (95%CI 1.13-2.40) for a concentration of < 50 nmol/L and 1.02 (95%CI 0.74-1.41) for participants with a concentration 50 to < 75 nmol/L compared to a concentration ≥ 75 nmol/L. After more extensive adjustment, the HRs were 1.28 (95%CI 0.86-1.90) and 0.91 (0.63-1.33) respectively. The fully adjusted HR per 10 nmol/L increase in 25(OH)D was 0.93 (0.86-1.01). The HRs did not vary by sex or between the three main racial/ethnic groups. As regression models were adjusted for more variables, the association diminished.

Table 13: Hazard Ratios for all-cause mortality by quartile of serum 25(OH)D from Ford *et al*., (2011)

		Model 1*	**Model 2§**	**Model 3†**	**Model 4‡**	**Model 5¶**
25(OH)D nmol/L	**Deaths**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**	**HR (95%CI)**
7-45	127/2362	1.74 (1.18-2.58)	1.24 (0.83-1.86)	1.57 (1.01-2.44)	1.39 (0.89, 2.17)	1.39 (0.90-2.14)
45-<60	93/1962	1.24 (0.83-1.86)	1.25 (0.84-1.85)	1.20 (0.80, 1.80)	1.13 (0.76-1.69)	1.12 (0.77-1.64)
60- <75	70/1708	0.93 (0.66-1.33)	0.92 (0.65-1.29)	0.90 (0.63-1.27)	0.85 (0.59-1.23)	0.83 (0.56-1.22)
≥ 75	57/1499	1.0	1.0	1.0	1.0	1.0

Model 1, adjusted for age and 6 month examination period, § Model 2 adjusted as model 1 and also for gender, race/ethnicity, †Model 3 adjusted as model 2 and also for educational status, smoking status, alcohol intake, leisure time physical activity, vitamin and mineral supplement use,, ‡ Model 4 adjusted as model 3 and also for blood pressure, a range of biochemical parameters (including serum Ca) and waist circumference, ¶ Model 5 adjusted as model 4 and also for history of cardiovascular disease and/or diabetes

As with earlier studies, this investigation was designed to establish whether low serum vitamin D was associated with an increased risk of all-cause mortality, but as with those considered by IOM (2011) there was a suggestion that the beneficial effect diminishes at high serum levels of 25(OH)D, the HR being higher in the top quartile than the third.

78. Zittermann *et al*., (2012) carried out a meta-analysis to examine the relationship between vitamin D deficiency and mortality. The studies analysed, which included those by Visser *et al*. (2006); Jia *et al*. (2007), Melamed *et al*. (2008) and Semba *et al*. (2009) that are described above, were observational investigations with reported RRs or crude data on overall mortality by serum 25(OH)D levels that

were sufficient for meta-analysis. Excluded were studies conducted in groups not relevant to the general population, and in individuals with disorders that could affect vitamin D metabolism such as chronic kidney disease. In the analysis of highest vs lowest categories of 25(OH)D, the summary relative risk (RR) for mortality was 0.71 (95%CI 0.50-0.91). In a parametric model, the estimated summary RRs (95% CIs) for mortality were 0.86 (0.82-0.91), 0.77 (0.70-84) and 0.69 (0.60-0.78) for individuals with an increase of 12.5, 25 and 50 nmol/L serum 25 (OH)D respectively, above a median "reference category" of approximately 27 nmol/L. However, there was no significant reduction in mortality when serum levels were 87.5 nmol/L above the reference category. The model used indicated a U-shaped dose-response, and the authors concluded that the optimal serum concentration was 75-87.5 nmol/L.

Conclusions on outcomes other than hypercalcaemia

79. Whereas the hazard of hypercalcaemia from over-exposure to vitamin D is well established, evidence of other adverse effects is less convincing. While some studies have suggested an increased incidence of falls and fractures following supplementation with vitamin D or with higher serum levels of 25(OH)D, the observation has been inconsistent, and there is no clear candidate biological mechanism that would account for such an effect. Likewise, findings on total mortality and risk of pancreatic and prostatic cancer in people with high serum levels of 25(OH)D have been contradictory, and where elevations of risk have been observed, they may have been a product of unrecognised residual confounding.

Toxicity in laboratory animals

80. A number of studies of vitamin D toxicity have been conducted in laboratory animals. In most investigations, hypercalcaemia and calcification of soft tissues were observed at high doses, with clinical signs such as anorexia, weight loss, weakness, lethargy, polyuria and polydipsia. These experimental findings are consistent with the case reports of human toxicity. In reproductive studies, adverse effects such as fetal loss, reduced fetal growth and bone lesions occurred only at doses causing maternal toxicity or significant disruption of calcium and phosphate homeostasis in the mothers. The available data suggest that rodents tolerate high intakes of vitamin D better than other species, including humans. As adequate human data were available, the data from animal studies were not used for quantitative risk assessment and are not discussed further in this statement.

Setting a TUL

81. A TUL (or UL) is equivalent to an Acceptable Daily Intake and is the maximum amount of a chemical that can be consumed every day over a lifetime without appreciable risk to health. TULs apply to specified population groups, but may not be protective of individuals within those groups who have identifiable medical disorders that render them unusually vulnerable to the chemical. They can be established using either human or animal data on adverse effects, and incorporate uncertainty factors as appropriate.

82. As noted above, sufficient human data are available on the adverse effects of vitamin D that they can be used to form a basis for risk assessment.

Critical health outcome

83. Of the well-established adverse effects of high intakes of vitamin D, that which occurs at the lowest doses is hypercalcaemia. Evidence for other possible effects which might occur at lower exposures, such as increased risks of kidney stones, falls, pancreatic cancer, prostate cancer and all-cause mortality, is less convincing and consistent. Thus the COT judged that hypercalcaemia should be the critical outcome on which to base TULs for vitamin D.

Key studies

84. The key study used by IOM to establish an UL was by Heaney *et al*., (2003), whereas EFSA used both the study by Heaney *et al*., (2003), and another by Barger-Lux *et al*., 1998, when setting a TUL.

85. The study by Heaney *et al*., (2003) was designed to investigate the relationship between steady state vitamin D_3 intake and serum 25(OH)D concentration, and to determine the proportion of the daily requirement that was met by tissue reserves. Doses of 0, 25, 125 or 250 µg/day were given to 67 healthy men for 20 weeks over the winter in Omaha, US. From a mean baseline value of 70 nmol/L, 25(OH)D concentrations increased in proportion to dose. The data reported on changes in serum calcium levels were limited, but indicate that among the 31 men in the top two dose groups, changes were in either direction (increase or decrease), and that no individuals had calcium levels above the normal reference range after treatment. The IOM noted that vitamin D intakes of 125 µg/day achieved serum 25(OH)D levels of 100-150 nmol/L (but not exceeding 150 nmol/L) after 160 days of administration.

86. The study by Barger-Lux *et al*., (1998) was designed to investigate the relationship between oral dosing and changes in circulating levels of vitamin D_3. Groups of 10-14 healthy men were given doses of 25, 250 or 1250 µg/day for 8 weeks. These doses resulted in mean ± SEM increases in serum 25(OH)D of 28.6 ± 5.3, 146.1 ± 12.0, and 643.0 ± 42.7 nmol/L above the baseline level for the whole study group of 67 ± 25 nmol/L. Limited data on serum calcium were reported, but from a mean group baseline of 2.41 ± 0.07 mmol/L (mean ± SD), no statistically significant changes were detected.

87. As noted previously, both IOM and EFSA established reference values of 100 µg/day vitamin D. IOM took the dose of 125 µg/day from the study by Heaney *et al*., (2003) as a NOAEL, but set the UL at 100 µg/day, a level "20% below the level identified by Heaney *et al*.," which reflected the uncertainties surrounding the data and the use of a single report. Using both the Heaney *et al*., (2008) and Barger-Lux *et al*., 1998 studies, EFSA identified a NOAEL of 250 µg/day, and applied a factor of 2.5 to account for uncertainties surrounding inter-individual variations in sensitivity to adverse effects of vitamin D in the long-term, and the TUL being based on only two studies in small samples of young men with minimal sun exposure.

88. COT did not identify any additional studies which indicated an increased risk of hypercalcaemia at lower doses than the NOAEL of 250 µg/day from the studies by Heaney *et al*., (2008) and Barger-Lux *et al*., (1998), and agrees that 100 µg/day is an appropriate TUL for adults. This TUL does not distinguish between total and supplementary vitamin D intake since dietary intakes of vitamin D are low and at most would make only a small contribution to total exposures at the TUL.

TULs for other population groups

89. Some groups might be more sensitive to high intakes of vitamin D than the general adult population. These are considered below.

Infants and children

90. A disorder termed idiopathic infantile hypercalcaemia was first recognised in the 1950s when a small number of infants presented with failure to thrive, vomiting, dehydration, spikes of fever and nephrocalcinosis (reviewed by Schlingmann *et al*., 2011). Laboratory investigations revealed severe hypercalcaemia with suppressed PTH, and in some cases the illness was fatal. The outbreak was attributed to increased doses of vitamin D (up to 100 µg/day) from infant formula and fortified milk. However, since most infants were unaffected, this could not be the only determinant. It was suggested that an intrinsic hypersensitivity to vitamin D might be involved, although it was unclear whether this was due to excessive activation of vitamin D or defective inactivation.

91. In 1957, the British Joint sub-committee on Welfare Foods recommended that the levels of vitamin D used to fortify National cod liver oil concentrate and National dried milk powder be reduced, and that the manufacturers of infant cereals should lower the average vitamin D content of those foods. The vitamin D content of evaporated milk products was also reduced. A study by Bransby *et al*., (1964) found that in 1960, vitamin D intakes of normal infants ranged from 6.25- 30 µg/day which was substantially lower than in the 1950s, when intakes of 100 µg/day were estimated to have occurred. It was further noted that the incidence of hypercalcaemia in infants had almost halved, with little or no increase in the occurrence of rickets. Occasional case reports of infantile hypercalcaemia have been published since, but these have related to specific genetic polymorphisms and are considered below in paragraph 110.

92. Early studies (Jeans and Stearns, 1938) suggested that excess vitamin D could reduce linear growth in infants, but in later research (Fomon *et al*., 1966) this was not observed at doses of up to 54 µg/day vitamin D/day. The absence of effect was supported more recently, by a large prospective study (Hyppönen *et al*., 2008), which monitored growth in 10,060 Finnish children supplemented with 50 µg/day vitamin D. Nor was growth affected in breast-fed children whose mothers were given 25 or 50 µg/day vitamin D from birth (Ala-Houhala *et al*., 1986). Where calcium levels were measured in these studies, they were unaffected by the supplementation.

93. A number of investigations have been conducted in babies and infants to explore whether supplementation could improve vitamin D status. These include the study by Ala-Houhala *et al*., (1986), and others by Vervel *et al*. (1997); Zeghoud *et al*., (1997) and Gordon *et al*., (2008). Various regimens of vitamin D supplementation were used, the highest dose being 1250 µg twice weekly for 6 weeks, but treatment-related hypercalcaemia was not observed.

94. Fewer data are available for older children, but treatment-related hypercalcaemia was not observed in groups of 8/9 children aged 10-17 years who received 350 µg/week vitamin D_3 in oil or ethanol for 8 weeks (Maalouf *et al*., 2008). Similar findings were reported in a second study by the same group (El Hajj Fuleihan *et al*., 2006), in which 340 healthy girls and boys received weekly doses of 35 or 350 µg vitamin D_3 or placebo for one year.

95. The IOM recommended ULs of 25 and 38 µg/day for infants aged 0- 6 and 6-12 months respectively. The studies on growth by Fomon *et al*., 1966 and Jeans *et al*., 1938 and the information about IIH were particularly important considerations in establishing these ULs. The EFSA panel (2012) retained a previous TUL of 25 µg/day established by the SCF in 2003 for children aged 0-12 months, taking into account in particular, the studies by Fomon *et al*., 1966, Jeans *et al*., 1938 and Hypönnen *et al*., 2011.

96. COT agrees with the TUL of 25 µg/day vitamin D for infants aged up to I year set by EFSA.

97. IOM (2011) commented that no data were available for specific age groups other than adults and infants. The IOM committee chose to scale down the adult UL of 100 µg to 62.5 µg/day for children aged 1-3 years and 75 µg/day for children aged 4-8 years to be consistent with the principle of graded tolerance with age. For children and adolescents aged 9-18 years, the ULs were the same as those for adults. Using the data from Maalouf *et al*., (2008) and El-Hajj Fuleihan *et al*., (2006) and considering it unlikely that adolescents in the phase of rapid bone formation would have a lower tolerance for vitamin D than adults, EFSA proposed a TUL of 100 µg/day for children aged 10-17 years. They noted that no new data had become available for children aged 1-10 years, but again considered it unlikely that children in a phase of rapid bone formation would have a lower tolerance of vitamin D than adults, and taking into account their smaller body size, agreed a TUL of 50 µg/day.

98. COT considers that the TULs of 50 and 100 µg/day vitamin D that were set by EFSA for children aged 1-10 and 11-17 years are appropriate.

Older adults

99. Renal insufficiency occurs in a substantial number of older adults (30% in North America cited in IOM, 2011), and might make them more sensitive to high intakes of calcium or vitamin D. Reduced renal function impairs calciuric responses. Conversely, renal failure also impairs metabolic activation of vitamin D in the kidney,

and patients with more severe disease may require treatment to counter this problem.

100. Another possible cause of vulnerability in the elderly is use of thiazide diuretics (which are prescribed for a sizeable proportion of older adults). Patients taking such medication are more challenged by high intakes of calcium and vitamin D because of a reduction in calcium excretion by the kidney.

101. However, the vast majority of vitamin D supplementation trials have been conducted in older adults, some of whom were frail and/ or were living in residential care. This suggests that any increased sensitivity in the elderly would have been reflected in their findings.

102. Although IOM considered the age groups, 51-70 and >70 years, they did not modify the UL for them (the reason for this was not discussed in their report).

103. Similarly, the EFSA panel did not make any adjustments to their TUL for older adults.

104. The COT concurs that although there are factors such as reduced renal function which might render older adults more sensitive to vitamin D, there are no indications from the available empirical data that a lower TUL should be specified for the elderly.

Pregnancy and lactation

105. Data on the effects of excess vitamin D in pregnancy are limited. However, a few studies have investigated vitamin D supplementation during pregnancy, as a means of improving the vitamin D status of both mothers and their babies. Serum calcium has not always been measured in such studies. Where it was done, hypercalcaemia was not observed, but the doses of vitamin D used were generally modest with only Hollis and Wagner (2004), Wagner *et al*, (2006) and Hollis *et al*., (2011) employing doses of ≥100 µg/day. No adverse effects were observed in these studies. The findings from trials of vitamin D during pregnancy are summarised in Table 14.

106. Similarly, there are few data on the effects of supplementing breast-feeding mothers with vitamin D. Studies have generally been designed to investigate ways of improving vitamin D status, and serum calcium has not always been measured. Where it has been done, hypercalcaemia was not observed (Table 15)

107. Neither IOM nor EFSA adjusted their UL/TUL to take account of pregnancy or lactation. COT agrees with this position.

Table 14: Summary of data from vitamin D supplementation trials in pregnant women.

Participants	**Dose (µg) and Duration of vitamin D**	**Baseline serum Ca mmol/L**	**Serum Ca after treatment mmol/L**	**Serum 25 (OH) D before treatment nmol/L**	**Serum 25 (OH)D after treatment nmol/L**	**Other adverse effects assessed or reported**	**Reference**
350 women (black, white & Hispanic) 12-16 wks gestation Charleston, US	10 50 100 D_3/day Until delivery	No difference between groups[5]	No effect of treatment	61.6 ± 27.1 58.3 ± 22.3 58.2 ± 21.8 mean ± SD	78.9 ± 36.5 98.3 ± 34.2 111.1 ± 40.4 Significant (p< 0.0001 by ANOVA).	No hypercalcaemia or hypercalciuria, no adverse events reported. High dropout rate	Hollis *et al*., 2011
30 women/group (non-pregnant controls) Turkey	10 D_3/day + 1000 mg Ca, or No treatment (pregnancy and post-partum)		2.07 ± 0.3 (post-partum) 2.11±0.09	Controls = 26.9 ± 17.7	47.8 ± 18.4 (1st trimester) 17.4 ±11.3 (postpartum)		Haliloglu *et al*., 2011
Groups of 21, 27, 29 women. France	25 D_2/day for last 3 months 5000 D_2x1 at 7 months Controls		2.15 ± 0.09 (2.44 ± 0.14) 2.15 ± 0.11 (2.41 ± 0.20) 2.10 ± 0.11 (2.37 ± 0.11) mean± SD maternal (cord) . No		25.3 ± 7.7 (15.7 ± 5.1) 26.0 ± 6.4 (18.2 ± 5.2) 9.4 ± 4.9 (5.3 ± 2.5)	No differences in birth weights or maternal calcium excretion	Mallet *et al*., 1986

[5] Limited data provided

Participants	Dose (µg) and Duration of vitamin D	Baseline serum Ca mmol/L	Serum Ca after treatment mmol/L	Serum 25 (OH) D before treatment nmol/L	Serum 25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
			differences between groups.				
40 women at end of 1st trimester France	25 D_3/day for last trimester, or controls	2.25[6] (1.05) 2.3 (1.1) Total (ionised)	2.28 (1.03) 2.23 (1.03) <u>Infants day 4</u> Vit D 2.28±0.5 (1.25 ± 0.1) Control 2.1±0.0 (0.98 ± 0.03) Mean ± SEM	27.5 25	62.4 27.5		Delvin *et al*., 1986
Indian Asian Middle Eastern, Black and Caucasian women, 60/group UK	1 x 5 mg D_2 dose 20 µg D_2/ day-wk 27 to delivery or, Placebo	within normal range	within normal range	25 (21-38) 26 (20-37) 26 (21-41) Median (IQR)	34 (30-46) 42 (30-46) 27 (27-39) <u>Cord</u> 17 (14-22) 26(17-45) 25 (18-34)	No differences in birth outcomes	Yu *et al*., 2009
British Asian women	25 µg D_2/day-last	2.42 ± 0.01	2.58 ± 0.02	20.1 ± 1.9	168.5 ± 12.5	Hypocalcaemia in controls	Brooke *et al*., 1980

[6] Maternal serum calcium and 25(OH)D values estimated from figure

Participants	Dose (µg) and Duration of vitamin D	Baseline serum Ca mmol/L	Serum Ca after treatment mmol/L	Serum 25 (OH) D before treatment nmol/L	Serum 25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
	trimester or, Placebo	mean± sem	2.51± 0.01 Cord blood 2.71± 0.02 2.65 ± 0.02		16.2 ± 2.7 Cord blood 137.9 ± 10.8 10.2 ± 2.0		
200 women (25/group analysed) India	30 µg /day + 375 mg Ca 20-24 wks-birth or, Placebo		2.32 ± 0.21 2.18 ± 0.24 Mean± SD				Marya *et al*., 1987

Table 15: Summary of data from vitamin D supplementation trials in lactating women.

Participants	Dose (µg) and Duration of vitamin D	Baseline serum Ca mmol/L	Serum Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
18 women <1 month post-partum and their breast-fed infants US.	40 90 D_2/day for 3 months. + a 10 µg D_3 multi-vitamin supplement		Within normal range.	68.9 ± 8.2 (19.7 ± 2.8) 82.1 ± 6 (33.4 ± 8.2) Maternal (infant) Mean ± SEM	90.1 ± 5.7 (87.4 ± 9.7) 111.1 ± 9.7 (76.9 ± 12.5) Maternal (infant) Mean ± SEM	No vitamin D related adverse effects observed. No hypercalcuria measured.	Hollis and Wagner, 2004

Participants	Dose (µg) and Duration of vitamin D	Baseline serum Ca mmol/L	Serum Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
19 women <1 mo post-partum and their breast-fed infants US.	150 D_3/day or placebo for 6 months. + a 10 µg D_3 multi-vitamin supplement Infants of placebo mothers also got 7.5/day supplement	2.3 ± 0.1 (2.6 ± 0.1) 2.3 ± 0.3 (2.5 ± 0.1) Maternal (infant) Mean ± SEM	2.4 ± 0.1 (2.6 ± 0.1) 2.4 ± 0.1 (2.5 ± 0.1) Maternal (infant) Mean ± SEM	84.9[7] (34.9) 80.4 (32.5) Maternal (infant) Mean ± SEM	95.9 (107.3) 146.7 (114.8) Maternal (infant) Mean ± SEM	No vitamin D related adverse effects observed. No hypercalcuria measured.	Wagner *et al*., 2006
Groups of 16-17 women post-partum	50 25 or 0 D_3/day for 15 weeks Infants of placebo mothers also got 10/day supplement	2.3 (2.7) 2.4 (2.6) 2.3 (2.6) Maternal (infant)	2.4 (2.5) 2.4 (2.5) 2.3 (2.5) Maternal (infant)	30 (22.5) 25 (12.5) 30 (12.5) Maternal (infant)	118 (44.9) 74.9 (34.9) 30 (87.4) Maternal (infant)		Ala-Houhala[8] *et al*., 1986

[7] 25(OH)D levels estimated from figure.
[8] Calcium and 25(OH)D estimated from figure.

Groups in which the TULs may not be protective

108. While a TUL need not apply to individuals in a population with specific diseases which render them more sensitive to a chemical, it is important to be aware of those who might not be adequately protected because of specific medical disorders.

Renal failure

109. Individuals with renal failure have been advised to use vitamin D cautiously (Medline plus, 2014, specific reference not given) as it could increase serum calcium levels, leading to a risk of arteriosclerosis. A Cochrane review of supplementation with vitamin D in patients with chronic kidney disease that did not require dialysis indicated an increased risk of hypercalcaemia (Palmer, 2009a). However, the compounds investigated were active forms of the vitamin including $1,25(OH)_2D$ and 1-α hydroxyvitamin D, and not vitamin D_2 or D_3. Similarly, a review of studies in patients with chronic kidney disease requiring dialysis (Palmer 2009b) indicated an increased risk of hypercalcaemia, but again, the treatments used were active forms of the vitamin and not vitamin D_2 or D_3.

Genetic polymorphisms

110. A number of genetic polymorphisms can affect the metabolism of vitamin D, and/or regulation of calcium. They include variations in the calcium-sensing receptor, vitamin D binding protein and the vitamin D receptor, and can alter calcium absorption and the "set point" of the calcium-sensing receptor, although it is unclear to what extent they modify responses to vitamin supplementation.

111. The best documented genetic polymorphism in vitamin D metabolism relates to the de-activating enzyme, CYP24A1, and prevents the breakdown of $1,25(OH)_2D$. This polymorphism has been linked with cases of idiopathic infantile hypercalcaemia (IIH), and various mutations in the gene have been reported. A number of cases of IIH have been described that occurred in response to vitamin D supplementation (reviewed Schlingmann *et al*., 2011 also Fencl *et al*., 2013). Although IIH cases are more usually reported in infants, three cases in adults have also been recorded (Tebben *et al*., 2012; Streeten *et al*., 2012). A further case was diagnosed in infancy but the patient presented again as an adult (Meusberger *et al*., 2013). Although the numbers of individuals involved are few, the condition appears to be milder in adults. The available case reports are tabulated below:

Table 16 Case reports of IIH

Patient	**Supplemental dose vitamin D (µg)**	**Reference**
6 patients aged 6-8 months 4 patients aged 5 wk-7 months	125/day D_3 from birth 15,000 D_3 1-3 doses	Schlingmann *et al*., 2011
Infant aged 4 months	16.5/day D_3	Fencl *et al*., 2013
Infant aged 10 months	Unknown	Dauber *et al*., 2012
Male aged 44 years	Unknown	Streeten *et al*., 2012

Male aged 39 years	Unknown	Tebben *et al*., 2012
Infant aged 3 months (presented again as an adult aged 29 years)	20/day D_3	Meusberger *et al*., 2013

Asymptomatic normocalcaemic primary hyperparathyroidism.

112. Primary hyperparathyroidism is a disorder of mineral metabolism characterised by incompletely regulated, excessive secretion of PTH from one or more of the parathyroid glands. It is the third most prevalent endocrine disorder and is most frequent in post-menopausal women (Fraser *et al*, 2009). The prevalence of primary hyperparathyroidism has been estimated to be 1/1000, 4.3/1000 and 3/1000 in the US, Sweden and Norway respectively but may be higher in some other populations (for example Fraser *et al*., cites a rate of 22/1000 reported in a Finnish population aged 55-75 years).

113. Primary hyperparathyroidism is frequently asymptomatic, often being diagnosed following an incidental finding of hypercalcaemia. However, it is now recognised that consistently elevated PTH levels may occur in association with normal serum levels of total and ionised calcium, in a "forme fruste" (mild, attenuated form) of the condition These normocalcaemic patients differ from those with mild hypercalcaemia who are occasionally normocalcaemic, as they are always normocalcaemic (Bilezikian and Silverberg, 2010).

114. Vitamin D deficiency may be associated with elevated PTH and normal serum calcium; in which case, correction of the vitamin D deficiency should normalise PTH levels. However, occasionally, when vitamin D deficiency is corrected, a normocalcaemic patient becomes hypercalcaemic and presents with typical clinical features of hypercalcaemic hyperparathyroidism. The asymptomatic condition is part of the spectrum of primary hyperparathyroidism (Eastell *et al*., 2009), but in order to make the diagnosis, other causes of secondary hyperparathyroidism should be excluded.

115. In a study by Maruani *et al* (2003) of 178 people with primary hyperparathyroidism, 34 had normal serum calcium levels and 144 had hypercalcaemia. The patients with normocalcaemia had, on average, lower levels of PTH than those with hypercalcaemia. They also had lower fasting urinary calcium excretion and renal tubular calcium reabsorption. In addition, normocalcaemic patients differed in having lower values for markers of bone turnover and plasma concentrations of 1,25 $(OH)_2D$, and higher renal phosphate thresholds[9].

116. The prevalence of asymptomatic hyperparathyroidism in the general population is uncertain. However, it might explain the sporadic occurrence of hypercalcaemia that has been reported in vitamin D intervention trials. Hypercalcaemia is considered in more detail in paragraphs 41-42.

[9] Renal phosphate threshold is the assessment of renal phosphate reabsorption under differing circumstances, allowing the evaluation of renal phosphate transport. It is the maximum rate of tubular reabsorption divided by the glomerular filtration rate.

Granulomatous disease

117. Granulomas are compact, centrally organised collections of epithelioid macrophages which may be encircled by lymphocytes. They may form in response to pathogens, for example the tubercle bacillus, or as part of a delayed hypersensitivity response to unknown antigens (for example, in sarcoidosis) or exposure to metals (for example, beryllium). Macrophages subject to chronic cytokine stimulation differentiate into epithelioid cells, gain secretory and bactericidal capability, lose some phagocytic capacity and fuse to form giant cells. In more mature granulomas, fibroblasts and collagen encase the ball-like clusters, and in some cases scarring ensues, altering organ architecture and function (Iannuzzi *et al*., 2007).

118. Hypercalcaemia has been described in almost all granulomatous diseases, including sarcoidosis, tuberculosis (TB), berrylliosis (chronic beryllium disease) and leprosy (Nayak-Rao, 2013). It occurs because the macrophages in granulomas produce 1-α hydroxylase which converts 25(OH)D to active $1,25(OH)_2D$. This activation is not regulated by PTH as it is in the kidney. Moreover, the enzyme is less sensitive than renal 1- α hydroxylase to product inhibition by $1,25(OH)_2D$. In addition, inflammatory mediators such as interleukin 2 may stimulate the further production of $1,25(OH)_2D$.

Sarcoidosis

119. Sarcoidosis is a rare disease which causes granulomas to develop in various organs of the body, most often the skin or lungs (NHS choices, 2013). It usually develops first early in adulthood, although in Scandinavia a bimodal incidence by age has been observed in women, with a first peak at 25-29 years and a second peak at 65-69 years (Iannuzzi, 2007). The highest annual incidence has been reported in Northern Europe (5-40 cases per 100,000 people), with a lower rate in Japan (1-2 cases per 100,000 people). Symptoms include tender red bumps on the skin, persistent cough, shortness of breath, fatigue, weight loss and night sweats.

120. Hypercalciuria is estimated to occur in 40% of patients, hypercalcaemia in 11% and renal calculi in 10% (Iannuzzi, 2007). Intra-renal deposition of calcium may be so severe that renal failure ensues. Higher estimates (2-63%) of the incidence of hypercalcaemia in sarcoidosis patients have also been reported (Sharma, 1996).

121. Several case reports have suggested that dietary vitamin D and/or exposure to sunlight can precipitate hypercalcaemia in people with sarcoidosis (Harrell and Fisher, 1939; Demetriou *et al*, 2010; Hassler *et al*., 2012; Amrein *et al*., 2011; Nayak-Rao, 2013).

Tuberculosis

122. Hypercalcaemia occurs in some cases of tuberculosis although the precise frequency of this is unclear (Isaacs *et al.,* 1987). For example, in a study by Roussos *et al*., (2001) mean (± SD) albumin-adjusted serum calcium concentration and serum ionised calcium concentration were significantly higher in patients with tuberculosis (2.49 ± 0.21 mmol/L and 1.27 ± 0.02 mmol/L) than in controls (2.36 ± 0.11 mmol/L and 1.19 ± 0.02 mmol/L). Hypercalcaemia was detected in 22 patients

with tuberculosis (25%), but only three had related symptoms. Similarly, corrected serum calcium was significantly higher in Chinese patients with untreated pulmonary tuberculosis than in matched controls (2.33 ± 0.07 vs 2.20 ±0.09 mmol/L), despite lower calcium intakes (Chan *et al*., 1996). However, there were no significant differences between the groups in serum levels of 25(OH)D or $1,25(OH)_2D$.

123. A study by Narang *et al*., (1984) explored whether vitamin D might have a role in the treatment of tuberculosis. One hundred and fifty patients were divided into five groups of 30 individuals according to the severity of their disease, and were studied along with healthy controls and controls who had chronic obstructive pulmonary disease. Within each group, participants were assigned to receive doses of 10, 20, 30, 60 or 95 µg of vitamin D/day for 3-6 months. Serum calcium was normal in all groups at baseline, but was non-significantly lower in the active tuberculosis group. It increased in all groups following treatment with all doses of vitamin D. The rise was significant in patients with active tuberculosis at all vitamin D doses, but in the control groups it was significant only at doses of 60 µg/day or greater, and did not lead to hypercalcaemia (≥2.97 mmol/L). In contrast, 19 of the 30 patients with active tuberculosis developed hypercalcaemia, though of these, only two experienced symptoms (nausea, vomiting and abdominal pain). The mechanism underlying the hypercalcaemia was unclear, but the authors concluded that anti-tubercular therapy should not be supported with vitamin D as patients appeared to be particularly sensitive to it. The finding is consistent with the known increase in 1 α-hydroxylase activity in granulomatous disease (IOM, 2011).

124. Sharma *et al*. (1981) reported hypercalcaemia in 10 out of 94 Indian patients with tuberculosis, and that these 10 patients were receiving a higher level of vitamin D supplementation than those who were normocalcaemic. A correlation was noted between daily vitamin D intake and the degree and duration of the hypercalcaemia. There was no indication of hypercalcaemia in matched controls with chronic obstructive pulmonary disease.

125. In contrast, in a small study by Fuss *et al*. (1988)[10], in which 11 patients with tuberculosis were given 50 µg 25(OH)D/day, corrected serum calcium and $1,25(OH)_2D$ levels were lower following treatment without any change in vitamin D binding protein levels. The authors concluded that hypercalcaemia was unusual in tuberculosis.

126. In a randomised double blind placebo-controlled trial by Wejse *et al*. (2009), patients with tuberculosis in Guinea-Bissau were given 2500 µg vitamin D or placebo at the start of treatment, and at 5 and 8 months. Clinical severity score and 12 month mortality were unaffected by the vitamin D treatment. Mild hypercalcaemia was reported both with vitamin D and placebo, and at 8 months, mean serum calcium levels were slightly higher in the treated group (2.17 vs 2.19 mmol/L).

127. In the UK, Martineau *et al.* (2011) conducted a randomised double blind placebo-controlled trial to investigate the effect of high dose vitamin D during the intensive phase of anti-microbial treatment for pulmonary tuberculosis. The patients received 2500 µg vitamin D at baseline, and 14, 28 and 42 days after starting

[10] Abstract only available.

standard treatment. The treatment increased serum levels of 25(OH)D but did not affect sputum conversion time. Mean serum calcium decreased during the study, but the rate of decline was not affected by treatment allocation. Mild hypercalcaemia was reported in two individuals (serum concentrations of 2.68 and 2.72 mmol/L corrected for protein) after two doses of 2500 µg vitamin D_3, but hypercalcaemia did not occur among the controls. It was suggested that the decline in mean serum calcium could have resulted from a reduction in granulomas in patients responding to treatment.

Other granulomatous diseases

128. Other granulomatous diseases may be associated with hypercalcaemia, including granulomatous myositis (Zhang *et al*., 2012) and disseminated giant cell polymyositis (Kallas *et al*., 2010). As with sarcoidosis and tuberculosis, this might be unmasked by low dose vitamin D supplementation.

Single or occasional doses of vitamin D

129. SACN asked that COT consider the possible effects of high single doses of vitamin D, which are sometimes used to improve vitamin D status in populations whose adherence to a schedule of more frequent lower doses might be poor. Several sources of information are relevant to this question.

130. As already described (see paragraph 55), Sanders *et al*., 2010 reported an increased frequency of falls and fractures in women aged ≥ 70 years who were given single, annual doses of 12,500 µg vitamin D, although they showed no evidence of hypercalcaemia.

131. Also, as noted previously, a number of case reports of vitamin D toxicity have been published (see Table 1). Although the toxicity may have resulted from multiple doses, the associated serum concentrations of 25(OH)D have implications for the effects of single doses. The reports suggest that vitamin D intoxication is generally associated with serum 25(OH)D levels > than 400 nmol/L and most often > 700 nmol/L. Thus, it appears that single doses which produce serum levels of 25(OH)D ≤ 300 nmol/L would be unlikely to cause hypercalcaemia.

Table 17: Changes in serum 25(OH)D levels following single doses of vitamin D

Dose (µg)	Population	25(OH)D Change from baseline (nmol/L)	Time of measurement (day)	Adverse effects reported/discussed	Reference
15,000 D_3	48 young people with vitamin D deficiency (25-56 y) Italy	+ 153 + 151 + 116	3 15 30	"Toxic" blood 25(OH)D levels (> 200 nmol/L) not reached.	Cipriani *et al*., 2010
15,000 D_2 or D_3	24 patients with vitamin D deficiency Italy	+ 15.22 + 30.95	30 30	No cases of hypercalcaemia, hypercalcuria or symptoms of vitamin D toxicity observed.	Cipriani *et al*., 2013a
15,000 D_3	18 young women with vitamin D deficiency Italy	+ 57.3 + 55.6 + 47.0 + 24.9 + 13.9	3 15 30 60 90	Not discussed	Cipriani *et al*., 2013b
15,000 D_2	43 infants East Germany	+ 400 (median)	15	34 % of infants had hypercalcaemia –few other details available	Markestad *et al*., 1987
12,500 D_3	2256 community dwelling women, ≥ 70y Australia	+ 70 + 40	1 mo 3 mo	Increased risk of falls and fractures	Sanders *et al*., 2010
12,500 12,500 + 1250/month	19, 22 elderly people (≥ 65 y) hospitalised at time of recruitment, largely independent thereafter.	+ 58 + 58 + 20 (estimated from Fig 2)	1 mo 1 mo 3 mo	Highest individual serum 25(OH)D was 220 nmol/L "within safe range" of 200-250 nmol/L.	Bacon *et al.,* 2009

Dose (µg)	Population	25(OH)D Change from baseline (nmol/L)	Time of measurement (day)	Adverse effects reported/discussed	Reference
	New Zealand				
7500 D_3	14/group elderly subjects with secondary hyperparathyroidism. Brazil	+ 50 + 37 +30	1 mo 2 mo 3 +mo	No hypercalcaemia measured. 2 cases of gastric intolerance noted.	Premaor *et al*., 2008
7500 D_2 Or D_3	8/group Elderly women Italy	79.87 62.4 43.28 ± 11.93 25.26 ± 16.85 94.85 104.83 119.38 ± 18.22 70.03 ± 20.8 (mean ± SD) or estimated from figures	3 7 30 60 3 7 30 60	Not discussed.	Romagnoli *et al*., 2008
7500 D_2	32 outpatients 49 Elderly in-patients (69-94y) New Zealand	+ 35 + 44 inpatients	Various but average of 17 weeks.	"no significant side effects". Serum calcium did not exceed reference range.	Wu *et al*., 2003
3750 D_3 given every 3 months or placebo	686 community dwelling women aged >70 y Subset of 40 analysed Australia	+ 37.44 higher than placebo overall	3, 6 and 9 months	No significant difference in adverse effects (including fracture) between groups.	Glendenning *et al*., 2010
2500 D_3	Elderly people in residential home	+ 62	2 weeks	25(OH)D "within normal limits".	Weisman *et al*., 1986. Abstract and proceedings[11].

[11] There appear to be 2 references for the same study, one being a conference proceedings.

Dose (µg)	Population	25(OH)D Change from baseline (nmol/L)	Time of measurement (day)	Adverse effects reported/discussed	Reference
2500 D_3	30 subjects (20 aged 61-84y and 10 aged 27-47y) US	+ 37 33 30 27 25 15	Measured at intervals, peak occurred at day 7, rest of values estimated from figure Day 14 21 30 35 60	25(OH)D levels did not reach those associated with toxicity.	Ilahi *et al*., 2008
1750 D_3	34 pregnant 27 non-pregnant women Bangladesh	32 28	Day 0, 2, 4, 7, and then weekly for up to 10 weeks. Different sampling schedules to minimise burden on volunteers.	No hypercalcaemia or supplement related adverse effects reported.	Roth *et al*., 2012
1250 of D_2, D_3 or placebo US	10 Healthy male volunteers/group (20-61y)	+ 12 + 17 -	Day 0, 1, 3, 5-7, 14 and 28.	Not discussed.	Armas *et al*., 2004

D_2 Ergocalciferol

D_3 Cholecalciferol

132. Intervention studies in which occasional large doses of vitamin D have been given and changes in serum 25(OH)D are summarised in Table 17. These show that mean serum 25(OH)D levels can increase substantially (up to about 100 nmol/L above baseline depending on dose, although usually less), but in only one study did they approach the levels found in case reports of toxicity. This was an investigation by Markestad *et al*., (1986) in which East German infants aged 1-20 months were given doses of 15,000 µg vitamin D_2 every 3 months. Median serum levels of 25(OH)D increased by up to 400 nmol/L two weeks after dosing, with increases in some individuals of up to 1000 nmol/L. Hypercalcaemia was observed in 34% of the infants. This finding is consistent with Cesur *et al*., 2003 in which there was a significant increase in hypercalcaemia in infants given a single dose of 15,000 µg vitamin D, being reported in 6/20 (30%) of the treated infants; serum 25(OH)D levels were not measured following vitamin D treatment in this study. With the exceptions of Sanders *et al*., 2010 and Markestad *et al*., 1986, no adverse effects were apparent in the tabulated studies, though it should be noted that only limited data are available.

Summary and discussion – single doses

133. The increased incidence of fracture and falls that was reported by Sanders *et al*., 2010 cannot be discounted, but there is no obvious toxicological mechanism that would explain a hazard of falls in the absence of hypercalcaemia, and the finding requires independent replication before it can be given much weight.

134. Results from most controlled studies in which occasional high doses of vitamin D have been administered suggest that serum levels of 25(OH)D would not reach the levels associated with vitamin D toxicity. An exception to this, however, is a study in infants aged 1-20 months, in which doses of 15,000 µg vitamin D_2 every 3 months increased serum concentrations of 25(OH)D by up to 1000 nmol/L and hypercalcaemia occurred in 34% participants (Markestad *et al*., 1987).

135. On the basis of this evidence, COT concludes that doses of 7500 µg at intervals of 3 months or longer would not be expected to cause adverse effects in adults. However, there is greater uncertainty about the effects of larger doses, which might cause hypercalcaemia in some individuals, even if only given infrequently. There are insufficient data to specify a safe upper limit for single doses in children, but the limited information that is available suggests that significant toxicity could occur in infants from a dose of 15,000 µg.

Overall summary

136. The Scientific Advisory Committee on Nutrition are reviewing dietary reference values for vitamin D and the COT were asked to consider the potential adverse effects of high intakes, both regularly over the long-term, and from single or occasional high doses of vitamin supplements.

Previous assessments

135. An extensive review of vitamin D was undertaken in 2011 by the US Institute of Medicine (IOM, 2011) which established an Upper Level (UL) of 100 µg/day vitamin D for adults. The IOM noted the paucity of long-term studies investigating the effects of vitamin D intakes above 250 µg per day or of maintaining serum 25-hydroxyvitamin D (25(OH)D) above 250 nmol/L. However, they considered it unlikely that symptoms of toxicity would be observed at vitamin D intakes below 250 µg/day, whereas adverse effects would be observed from consumption at or above 1250 µg/day over weeks or months.

136. In 2012, EFSA (2012) established a tolerable upper level (TUL, equivalent to the term UL used by the IOM) for adults of 100 µg vitamin D per day. A TUL is intended to apply to all groups of the general population, including more sensitive individuals, throughout life stages such as pregnancy, but with the exception in some cases of discrete, identifiable sub-populations who may be especially vulnerable to one or more adverse effects (e.g. those with unusual genetic predisposition, certain diseases, or receiving the nutrient under medical supervision).

137. The EFSA and IOM reviews were used as the initial bibliographic sources for the COT's review, with an updated and expanded literature search performed as required.

Vitamin D and calcium metabolism

138. Vitamin D occurs as ergocalciferol (vitamin D_2), which is produced by UV irradiation of plant and fungal materials, and cholecalciferol (vitamin D_3), which is formed in the skin when it is exposed to UV irradiation. The two forms of the vitamin appear to have broadly similar potency and toxicity. However, there is some suggestion that D_2 may be less toxic and also less effective at high doses.

139. Vitamin D requires metabolic transformation before it becomes biologically active. This entails sequential hydroxylations to form first 25 hydroxyvitamin D (25(OH)D), and then 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), which is the active form of the vitamin (IOM, 2011).

140. Vitamin D has a critical role in the metabolism of calcium and phosphate, which are essential for the mineralisation of bone. Its main actions are to increase absorption of calcium from the intestine and stimulate the mobilisation of calcium from bone. Production of active $1,25(OH)_2D$ in the kidney by the enzyme, 1α-hydroxylase, is regulated by parathyroid hormone (PTH) as part of a complex feedback system, and can also be stimulated by low serum levels of phosphate.

141. Serum concentration of 25(OH)D is the best indicator of a person's vitamin D status, since the active form, $1,25\ (OH)_2D$, has only a short half-life and its formation is modified by the level of PTH in response to changes in serum calcium and/or phosphate levels. In the UK, mean serum 25(OH)D concentrations were found to range from 41-47 nmol/L, with high level concentrations from 87.5-106 nmol/L.

Sources of vitamin D

142. In the absence of supplementation, most circulating vitamin D_3 is derived from its UV-dependent formation in the skin. In addition, vitamin D_2 is found in foods of non-animal origin (particularly fungi), and vitamin D_3 in foods such as fatty fish, fish liver oil and egg yolk. Also, some foods, such as milk, margarine and breakfast cereals, may be fortified with vitamin D.

143. In the UK, data from the NDNS rolling programme indicate mean dietary intakes of vitamin D (excluding supplements) in adults of about 3 µg/day and high level intakes of about 9 µg/day.

144. Single supplements may contain up to 250 µg vitamin D per daily dose, while most multi-vitamin supplements provide 5 µg vitamin D per day.

Vitamin D toxicity

145. Excessive intakes of vitamin D can cause various adverse health effects as a consequence of hypercalcaemia. This occurs through increased absorption of calcium from the gut and resorption of calcium from bone, and can result in deposition of calcium in soft tissues, diffuse demineralisation of bones, and irreversible renal and cardiovascular toxicity. Symptoms and signs may include anorexia, nausea, vomiting, weakness, lethargy, constipation and non-specific aches and pains, as well as thirst, polyuria, weight loss and cardiac dysrhythmias. These effects have been described in a number of case reports of vitamin D intoxication, and hypercalcaemia has also been reported in a few individuals taking part in trials of vitamin D supplementation.

146. Other possible adverse effects that have been linked with high intakes of vitamin D or high serum levels of 25(OH)D include an increased incidence of falls and fractures, increased rates of pancreatic and prostatic cancer, and elevated total mortality (i.e. from all causes combined). However, the evidence for these associations is less consistent and convincing than that relating to hypercalcaemia.

147. Data from studies in laboratory animals are largely consistent with the findings from case reports and studies in humans. In most investigations, hypercalcaemia and calcification of soft tissues have been observed. In reproductive studies, adverse effects such as fetal loss and impaired fetal growth have occurred, but only at doses causing maternal toxicity or significant disruption of calcium and phosphate homeostasis in the mothers.

TULs

148. The best established adverse effect of high intakes of vitamin D is hypercalcaemia and the COT judged that this should be the critical outcome on which to base TULs for vitamin D.

149. The two most informative studies relating to this endpoint (by Heaney *et al*., 2003 and Barger-Lux *et al*., 1998) indicate a NOAEL of 250 µg/day. Based on this point of departure, and applying an uncertainty factor of 2.5 (to account for inter-individual variations in sensitivity and the derivation of the NOAEL from only two studies in small samples of young men with minimal sun exposure), EFSA

established a TUL of 100 µg/day vitamin D for adults . The COT agrees with this value, which is also the UL for adults proposed by IOM.

150. EFSA established TULs of 25, 50 and 100 µg/day vitamin D for infants aged up to 1 year, and children aged 1-10 and 11-17 years respectively, taking into account several studies of supplementation in infants and children. The COT considers that these TULs also are appropriate. The TUL for infants aged up to 1 year is lower than the intakes that are thought to have precipitated an outbreak of idiopathic infantile hypercalcaemia (IIH) that occurred in the UK in the 1950s.

151. The TULs proposed do not distinguish between total and supplementary vitamin D intake since dietary intakes of vitamin D are low and at most would make only a small contribution to total exposures at the TULs.

152. No evidence was found that pregnant women or older adults were unusually sensitive to vitamin D, and therefore the TUL of 100 µg/day vitamin D for adults should be appropriate for these groups.

153. The TULs proposed might not, however, protect individuals with medical disorders that pre-dispose to hypercalcaemia. These include normocalcaemic hyperparathyroidism, granulomatous diseases such as sarcoidosis and tuberculosis and genetic pre-disposition such as occurs in IIH.

Single and/or occasional doses of vitamin D

154. A finding that the incidence of fractures and falls was increased in women given annual doses of 12,500 µg vitamin D requires independent replication before it can be given much weight. Results from most controlled studies in which occasional high doses of vitamin D have been administered suggest that serum 25(OH)D would not reach the levels associated with vitamin D toxicity, although in one study of infants aged 1-20 months, doses of 15,000 µg vitamin D_2 every 3 months increased serum concentrations of 25(OH)D by up to 1000 nmol/L and hypercalcaemia occurred in 34% of participants.

155. The COT concludes that doses of 7500 µg at intervals of 3 months or longer would not be expected to cause adverse effects in adults. However, there is greater uncertainty about the effects of larger doses, which might cause hypercalcaemia in some individuals, even if only given infrequently. There are insufficient data to specify a safe upper limit for single doses in children, but the limited information that is available suggests that toxicity could occur in infants from a dose of 15,000 µg.

Conclusions

156. The COT have drawn the following conclusions:

a) Excess vitamin D intake can result in hypercalcaemia, demineralisation of bone, soft tissue calcification and renal damage. This may result from both acute and chronic exposure. Hypercalcaemia is the most appropriate

endpoint on which to base TULs for vitamin D, since adverse effects that might occur at lower doses through other mechanisms have not been reliably established.

b) TULs of 100 µg/day vitamin D for adults and children aged 11-17 years, 50 µg/day for children aged 1-10 years, and 25 µg/day for infants, as recommended by EFSA, are appropriate. These TULs do not distinguish between total and supplementary vitamin D intake since dietary intakes of vitamin D are low and at most would make only a small contribution to total exposures at the TULs.

c) The TULs proposed may not provide adequate protection for individuals with medical disorders that pre-dispose to hypercalcaemia. These include normocalcaemic hyperparathyroidism, granulomatous diseases such as sarcoidosis and tuberculosis, and genetic pre-disposition such as occurs in idiopathic infantile hypercalcaemia.

d) Doses of 7500 µg at intervals of 3 months or longer would not be expected to cause adverse effects in adults. However, there is greater uncertainty about the effects of larger doses, which might cause hypercalcaemia in some individuals, even if only given infrequently. There are insufficient data to specify a safe upper limit for single doses in children, but the limited information that is available suggests that toxicity could occur in infants from a dose of 15,000 µg.

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Abbreviations

ATBC	Alpha Tocopherol Beta Carotene
BMI	Body Mass Index
Ca	Calcium
CKD	Chronic kidney disease
CI	Confidence Intervals
CLUE	Campaign against Cancer and Stroke
COMA	Committee on the Medical Aspects of food policy
COPD	Chronic Obstructive Pulmonary Disease
CRP	C Reactive Protein
CYP	Cytochrome P450
D_2	Ergocalciferol
D_3	Cholecalciferol
$1,25(OH)_2D$	1,25-dihydroxyvitamin D_2
DBP	Vitamin D Binding Protein
DRV	Dietary Reference Value
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EVM	Expert Group on Vitamins and Minerals
GFR	Glomerular Filtration Rate
GI	Gastrointestinal
HPFS	Health Professionals Follow up Study
HR	Hazard Ratio
25(OH)D	25-hydroxyvitamin D
IIH	Idiopathic Infantile Hypercalcaemia
IOM	Institute of Medicine
IRR	Incidence Rate Ratio
IU	International Units
Kcal	kilocalories
L	Litre
MED	minimal erythemic dose

mg/day	milligrams/day
μg/day	micrograms/day
MI	Myocardial Infarction
ml	millilitres
mmol/L	millimoles/Litre
mo	months
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NDNS	National Diet and Nutrition Survey
NHANES	National Health and Nutrition Examination Survey
NHS	National Health Service
ng/ml	nanograms/millilitre
nmol/L	nanomoles/Litre
OR	Odds Ratio
pg/ml	picograms/millilitre
PLCO	Prostate, Lung, Colorectal and Ovarian Screening Trial
PTH	Parathyroid Hormone
RCTs	Randomised Controlled Trials
RR	Relative Risk
SACN	Scientific Advisory Committee on Nutrition
SCF	Scientific Committee on Food
SD	Standard Deviation
SEM	Standard error of the mean
TB	Tuberculosis
TUL	Tolerable Upper Level
UK	United Kingdom
UL	Upper Level
US	United States
UV	Ultraviolet
VDR	Vitamin D Receptor
wk	week
y	Years

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Statement on adverse effects of high levels of vitamin D.

Annex 1

Search strategy

Members agreed to use the 2011, IOM report as a bibliographic source. Relevant references were taken from this document and from the EFSA panel report published in 2012.

An update search was carried out by the secretariat as below:

Scientific/medical name(s): 1,25 dihydroxycholecalciferol, 25 hydroxycholecalciferol, 1,25 dihydroxyvitamin D

Search 1:

“vitamin D” OR “vitamin D2” OR “vitamin D3” OR ergocalciferol* OR cholecalciferol*

AND intake* AND

“adverse health outcome*” OR “adverse health effect*” OR toxic*

Search 2:

serum OR “blood level*”

AND

“vitamin D” OR “25 hydroxyvitamin D” OR “25-hydroxy vit D” OR “1,25 dihydroxyvitamin D” OR “25-hydroxy vit D” OR “plasma vit D” OR 25OHD OR 25-OHD OR 25OHD3 OR “25(OH)D3” OR 25-OHD3 or “25-(OH)D3” OR “25(OH)D” OR “25-(OH)D” OR “25-OH-D” OR 25-hydroxycholecalciferol OR 25-hydroxyergocalciferol OR calcidiol OR calcifediol

AND

“adverse health effect*” OR “adverse health outcome*” OR toxic*

from Google Scholar; FoodlineWeb; PubMed; IngentaConnect; Thomson Reuters Web of Knowledge: ISI Web of Science

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT

Statement on adverse effects of high levels of vitamin D.

Annex 2

Tables summarising the supplementation studies considered by IOM and EFSA are summarised in the attached Tables.

Table A1 contains the RCTs identified in the systematic review conducted for IOM.

Table A2 contains additional studies considered by IOM

Table A3 contains studies considered by the EFSA panel.

Table A1. RCTs able to assess adverse effects as identified in the Ottawa review for IOM

Participants	**Dose (µg) and Duration of vitamin D**	**Baseline Ca mmol/L**	**Ca after treatment mmol/L**	**25 (OH) D before treatment nmol/L**	**25 (OH)D after treatment nmol/L**	**Adverse effects assessed or reported**	**Reference**
56 Infants (20,20 & 16/group) with vit D deficient rickets + 20 controls, mean age =10 months Turkey	3750 75000 15000 Single dose	1.96 ± 0.34 (patients with rickets) 2.22 ± 0.12 (controls) Mean ± SD	↑ 0.41 ± 0.29 ↑ 0.55 ± 0.44 ↑ 0.61 ± 0.49	0.016[12] ± 0.0063 0.015 ± 0.0047 0.015 ± 0.004		Hypercalcaemia in 2/20 at 7,500 and 6/20 at 15,000 µg 30 d after treatment. Ca/Cr ↑after treatment, persisting at top dose.	Cesur *et al*., (2003)
Healthy neonates 15, 15 and 30/group Algeria	2500 D_3 at 0, 3 and 6 mo, 5000 D_3 x1 at birth, 15,000[13] x1 at 15 d	2.35 ± 0.25 2.33 ± 0.28 2.2 ± 0.23 Mean ± SD	2.38 ± 0.08 2.38 ± 0.1 2.52 ± 0.13 at 0.5 mo, significantly increased (p <0.005) from baseline and lower doses	All < 25 nmol/L	92 ± 42 150 ± 55 307 ± 160	No hypercalcaemia but transient elevation in Ca in top dose.	Zeghoud (1994)
Female children and adolescents (55-58/group) Lebanon	Placebo 35 Vitamin D_3 350 Vitamin D_3/week for 1 year	2.5 ± 1 2.48 ± 0.8 2.48 ± 1 Mean ± SD		34.9 ± 17.5 34.9 ± 22.5 34.9 ± 19.9 Mean ± SD	40 ± 19.9 43 ± 14.9 95 ± 77 3/55 in top dose had >250	No hypercalcaemia in treatment group.	El-Hajj Fuleihan *et al*., 2006

[12] The values are converted from those in the paper given as pg/ml, although 25(OH)D is usually reported as ng/ml or nmol/L.
[13] 15 mg data from an earlier study (Markestad *et al*., 1987) but included in this paper

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Adverse effects assessed or reported	Reference
Healthy adults (28-33/group; no placebo) Canada	25 D_3 100/day vitamin D_3 0 (no supp) for 2-5 months		All within normal range (2.2-2.6) and no significant change from baseline.	40.7 ± 15.4 Mean (SD)	68.7 ± 16.9 96.4 ± 14.6 Plateau after 3 mo 46.7 ± 17.8 Controls (summer)	More incidences of elevated urinary calcium: creatinine in top dose but not significant.	Vieth *et al*., 2001
Adult endocrine outpatients, 53-55y (64-66/group to start) Some patients continued on to a 2nd study plus additional new patients Canada	Study 1 15 D_3 100 D_3 Study 2 15 D_3 100 D_3 for 2 months Overlapping design meant that the duration of dose was 2-15 mo	1.23[14]	1.23 1.235 122, 1.235 After 2 and 6 months treatment. No significant differences between groups or over time.	48 ± 9 39 ± 9	79 ± 30 112 ± 41	No hypercalcaemia	Vieth *et al*, 2004
208 post-menopausal African American women (104/group) Long Island, US	20/day vitamin D_3 for 2y then 50 µg/day for 3rd year or, placebo + Ca supplements to ensure Ca intake of 1200-	2.2	2.31 (both groups) at 2 years. 2.34 (controls) and 2.38 (treated) at 3 years. No statistical difference.	46.9 (95%CI 43.9-50.9)	70.8 (66.4-76.1) within 3 mo of treatment. Further increased to 86. (80.1-94.1) within 3 mo of dose increase.	6 mild hypercalcaemia in treatment group, 2 in controls. 3 elevated urinary Ca (but no difference in 24h urine Ca per kg bw) (placebo-92.0, 107.4, 100.6 and treatment 86.3, 118.8 and	Talwar *et al*., 2007 Aloia *et al*., 2005

[14] Data estimated from figure.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Adverse effects assessed or reported	Reference
	1500 mg/day		Mean serum Ca 0.062 mmol/L higher in top serum vitamin D quartile		No change in controls.	113 mg/day) at 0, 2 and 3y.	
Older women in residential care, mean age 85 (60-62/group) Switzerland	20 D_3/day + 1200 mg Ca placebo + 1200 mg Ca for 12 weeks	2.34 (2.28-2.39) 2.32 (2.23-2.39 Median (IQR))	2.34 (2.25-2.42) 2.28 (2.22-2.34) No significant difference.	30.7 (23.0-54.91) 28.95 (23.0-54.91)	65.4 (49.67-82.62) 28.5 (24.46-41.43)	No hypercalcaemia	Bischoff , 2003
48 women with osteoporosis or osteopenia (mean age 70)	20 D_3/day + 1000 mg Ca + 10 mg alendronate or placebo + 1000 mg Ca + 10 mg for 3 months	2.33 (2.21-2.39) 2.26 (2.22-2.37) Median (IQR)	2.30 (2.21-2.38) 2.29 (2.22-2.33)	22.46 (17.42-24.96) 19.97 (17.42-27.46)	64.9 (52.42-62.4) 34.95 (22.46-47.42)	No hypercalcaemia No hypercalcuria but urinary Ca/cr from baseline in treatment group by day 30	Brazier *et al*, 2002
192 women with vitamin D insufficiency (mean age 65 y) France	20 D_3/day + 1000 mg Ca or placebo For 1 year	2.19 2.17 Median	2.29 (2.19-2.39) 2.27 (2.19-2.42) Median (Q1-3). Not significant by *t* test	18.25 17.5	71.8 26.8	Hypercalcaemia 7/95 11/96 (controls) 24h urinary Ca/cr sig increased in treatment group (<0.001)	Brazier *et al*., 2005
3270 Healthy women in senior facilities (1,634-1,636 /group) Age 84 ± 6y	20 µg D_3/day + 1200 mg Ca or, placebo for 1.5 y	2.29 ± 0.09 2.29 ± 0.1 Mean ± SD	2.30 ± 0.08 2.25 ± 0.09 (sig lower than baseline)	40 ± 27.5 32.0 ± 22.47	105 ± 22.5 27. 5 ± 17.5	No hypercalcaemia or renal calculi. More GI effects in treatment group	Chapuy *et al*., 1992

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Adverse effects assessed or reported	Reference
France							
Healthy women in senior facilities with low Ca and Vit D intakes (190-194/group) France	20 D_3 + 1200 mg Ca (fixed combination) or 20 µg D_3 + 1200 mg Ca (separate) or, placebo/day for 2 y	2.31 ± 0.11 2.29 ± 0.12 2.3 ± 0.11 Mean ± SD	2.31 ± 0.12 2.32 ± 0.13 2.27 ± 0.13 Significantly higher in treated groups.	21.22 ± 13.23 22.46± 16.47 22.71 ± 17.22	75[15] 80 15 Significantly higher in treatment groups.	No hypercalcaemia (3 but unrelated to treatment). Non-significant increase in hypercalcuria More GI effects in treatment group but also not significant.	Chapuy *et al*., 2002
Elderly patients in hospital (mean age 82y) (41/group) UK	22.5 D_2/day or placebo up to 40 wks	2.32 ± 0.02 2.34 ± 0.02 Mean ± SEM		17.63 ±2.05 16.60 ±2.10	110[16] 17	1/41 hypercalcaemia (attributed to hyper-parathyroidism)	Corless *et al.,* 1985
Healthy older men and women (mean age 70-72y) (187-202/group completed) US	36 µg D_3 + 500 mg calcium/day or Placebo For 3 years	1.25 ± 0.05, 1.25 ± 0.05 (men, women) 1.25 ± 0.05, 1.28 ± 0.03 (men, women) Mean ± SD	↑0.03±0.05, ↑0.05±0.05, (men, women) sig (p< 0.005 for men only). ↑0 ± 0.03, ↑0 ±0.05, (men, women)	82.37 ± 40.7 71.64 ± 33.2 (men, women) 83.87 ±33.6 61.15 ± 25.7 (men, women	↑29.45 ± 29.0 ↑40.2 ± 35.7 (men, women) ↓6.7 ± 25.5 ↑1.7 ±20.2 (men, women)	No hypercalcaemia (small ↑ in serum Ca). 1hyper-calcuria, 24 h urinary Ca/cr increased	Dawson-Hughes *et al*., 1997

[15]Data taken from figure.
[16] Data taken from figure

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Adverse effects assessed or reported	Reference
Adults 1306-1343/group Scotland	20 D_3 /day or 20 D_3 /day + 1000 mg Ca or, 1000 mg Ca or, Placebo for 5 years			37.9 ± 16.2 (sample of 60)	↑24.2 ± 21.7 ↑24.5 ±17.2 ↑3.5 ± 14.2 ↑7.8 ± 18	21 cases hypercalcaemia but no differences between groups. No differences in renal stones, GI effects, adverse events, renal insufficiency, mortality	Grant *et al.,* 2005 RECORD trial
Adults 36-39/group in hospital. Mean age 81.2y UK	20 D_2 + 1000 mg/d Ca (tablet) or, placebo for 1 year	2.35 (2.0-2.6) 2.39 (2.0-2.6) Mean (range)	2.42 2.40	29 (6-75) 30 (12-128)	50 27	No hypercalcaemia, no change in serum Ca.	Harwood *et al*., 2004.
Adults (18,106 or 18,176 /group)	10 D_3+ 1000 mg Ca or, placebo for 7 years				46 48.4	Increase in renal stones. Slight increase in GI effects.	Jackson *et al*., 2006
Elderly women. Free living (25 intervention, 27 controls)	45 D_3/day + 1558 mg Ca for 11 weeks (winter) or, no treatment	2.44 (2.3-2.6) 2.49 (2.4-2.6) Mean (95%CI)	2.40 (2.3-2.5) 2.41 (2.3-2.6)	Mean 38.5	80.7 23.3	Mild GI effects in 9/25 free living individuals. No change in creatinine levels.	Honkanen *et al*., 1990
Institutionalised (30 intervention, 33 controls) Finland	45 D_3/day + 1558 mg Ca for 11 weeks (winter) or, no treatment	2.59 (2.4-2.8) 2.56 (2.4-2.8) Mean	2.58 (2.4-2.8) 2.73 (2.5-2.9)	Mean 24.1	64.4 10.4		

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Adverse effects assessed or reported	Reference
		(95%CI)					
Adults, mean age ≥ 65y (32-33/group) Connecticut, US	50 D_3 D + 500 mg Ca/d or placebo (500 mg Ca) for 11 weeks			65 ± 16.7 58.9 ± 18.7	87.1 ± 13.7 56.4 ± 17	No hypercalcaemia or hyper-alcuria	Kenny *et al*., 2003
Elderly institutionalised women 124/group Switzerland	11 D_3 + 1000 mg calcium or placebo for 2 years	Mean ± SD	2.27 ± 0.09 2.27 ± 0.09,	29.7 ± 3 29.2 ± 3	↑123% ↓ 51 % 74.5 and 20.8 at 1 y and 66.3 and 14.3 at 2 y	1 hypercalcaemia 6 withdrawals in treatment group due to GI effects	Krieg *et al*., 1999. Abstract only
Institutionalised elderly men and women (81y senior home) 84 y nursing home) (70-72/group) Netherlands	10 D_3/day or 20 D_3/day for 1 year.				Increased to >40	1 hypercalcaemia unrelated to treatment non sig increase in Ca/cr and sig increase in serum creatinine.	Lips *et al*., 1988 Abstract only
Post-menopausal women aged 50-70y (12-13/group) Argentina	125 D_2 /day + 500 mg Ca or, 250 D_2 /day + 500 mg Ca or, 500 mg Ca for 3 months	2.33 (2.33-2.4) Both treatment groups Median (IQR)	2.4 (2.3-2.48). Both treatment groups. No individual value outside normal.	42.0 (23.7-45) 32.5 (27.5-45.0) 45.0 (31.2-61.2)	77.5 (66.2-56.2) 97.7 (79.3-123.1) 55.0 (72.5[17]-68)	No hypercalcaemia No difference in hyper-calcuria between groups	Mastaglia *et al.,* 2006

[17] As given in paper

Table A2. Studies considered in main IOM (2011) report where no hypercalcaemia was documented.

Some studies reported instances of hypercalcaemia but these were considered to be unrelated to treatment

Participants	**Dose (µg) and Duration of vitamin D**	**Baseline Ca mmol/L**	**Ca after treatment mmol/L**	**25(OH)D before treatment nmol/L**	**25(OH)D after treatment nmol/L**	**Other adverse effects assessed or reported**	**Reference**
60 subjects. UK and St Louis, US	45 250, 500 1000 for 4wks. 35 40 150 220 500 1000 2000 3000 for ≥ 4 months	Unclear whether Ca was measured at any point.		7.5-60[18]	50 92.5 162.5 300 - 70 120 200 350 500 700 900		Stamp *et al*., 1977
449 Elderly subjects (in a review of 11 smaller studies)	10-20/day High dose 2500 /year					Hypercalcaemia in 3 (2/3 with pre-disposing cause)	Byrne *et al*., 1995
Elderly women. Free living (25 intervention, 27 controls)	45 D_3/day + 1558 mg Ca for 11 weeks (winter) or, no treatment	2.44 (2.3-2.6) 2.49 (2.4-2.6)	2.40 (2.3-2.5) 2.41 (2.3-2.6)	Mean 38.5	80.7 23.3	Mild GI effects in 9/25 free living individuals. No change in creatinine levels.	Honkanen *et al*., 1990

[18] 25(OH)D values estimated from figures.

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25(OH)D before treatment nmol/L	25(OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
		Mean (95%CI)					
Institutionalised (30 intervention, 33 controls) Finland	45 D_3/day + 1558 mg Ca for 11 weeks (winter) or, no treatment	2.59 (2.4-2.8) 2.56 (2.4-2.8) Mean (95%CI)	2.58 (2.4-2.8) 2.73 (2.5-2.9)	Mean 24.1	64.4 10.4		
109 subjects (> 60 or 65y) UK	40 µg /ml oil or oil placebo. Doses varied, up to 3000 µg vitamin D_2 or D_3 or up to 250 µg 25(OH)D Varying durations, some over 4 months	2.430 (2.110-2.650) 2.411 (2.241-2.481) Mean ± 2 SD	2.431 (2.111-2.651) 2.416 (2.246-2.486) No change in mean serum calcium, but increased (Mann-Whitney U test, p= 0.01) in treatment group when corrected			Hypercalcaemia in 2/63	Johnson *et al*., 1980
Patients on anti-convulsants England	250 µg/day for 10 weeks				110		Davie *et al*., 1982 Abstract only

Table A3. Additional studies considered by EFSA

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
200 Healthy overweight adults	83 D_3/day or placebo for 12 mo during weight loss programme.	2.36 2.38	2.38 2.40	30.0 ± 17.5 30.3 ± 20.1	85.5 ± 57.5 42.0 ± 35.0		Zitterman *et al*., 2009
150 subjects with TB, 30 groups (I-V) at different stages of infection. Further divided for dose (A-E) 6/group) + controls India	10 20 30 60 95 For 15 days,1, 2 and 3 months	A 2.4, B 2.38 C 2.38, D 2.43 E 2.46 (Controls)	2.5, 2.43, 2.66, 2.62, 2.83 P<0.02 in top dose groups			Hypercalcaemia where infection active	Narang *et al*., 1984
138 adults 19-65y Long Island, US	Dose 50-100 D_3 per day. Adjusted to achieved serum concentration in the range 75-220 nmol/L or placebo 3 winters	2.25 ± 0.17 2.22 ± 0.1 2.25 ± 0.1 2.3 ± 0.12 (female blacks, whites, male blacks, whites respectively). Mean ± SD	No values > 2.65	40.9 ± 14.4 57.3 ± 14.6 34.9 ± 16.4 59.9 ± 12.4 (female blacks, whites, male blacks, whites respectively).	Only 1 patient exceeded 200 nmol/L.	No hypercalcaemia observed, 4 patients had hypercalcuria on some occasions.	Aloia *et al*., 2008
Healthy adults	25		All within normal	40.7 ± 15.4	68.7 ± 16.9 (40-	More incidences of	Vieth *et al*.,

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
(28-33/group; no placebo) 25 comparable subjects Canada	100 /day D_3 0 for 2-5 months		range (2.2-2.6) and no significant change from baseline.	(mean ± SD)	100) 96.4 ± 14.6 (69-125) Plateau after 3 months 46.7 ± 17.8 Controls (summer)	elevated urinary calcium:creatinine in top dose but not significant.	2001
19 Healthy pre-menopausal (22-49y) women Denmark	100 D_2 /day or 100 D_3 /day 8 weeks	2.46 ± 0.03 2.46 ± 0.02 Mean ± SEM	2.46 ± 0.01 2.51 ± 0.02 P<0.02 by Mann Whitney test	75.1 (55.1 -95.6). 77.4 (46.2 -100.3).	88.6 (19.7-120.6) 113.3 (88.6-148.3) P<0.001 by Wilcoxon's test	Significant increase in urinary calcium excretion with D_3	Tjellesen *et al*., 1986
163 Healthy post-menopausal white women (20-21/group) Nebraska, US	10 20 40 60 80 100 120 D_3/day or, placebo for 1 year	2.37 (0.075) Mean (SD)		38.2 (9.4)	Modelled as curve, plateau at 112 nmol/L at 80-120 µg/day	1 1 1 1 5 0 4 2 1 0 1 0 2 1 1 0 Individuals with serum Ca ≥ 2.5 or ≥2.7, normalised after re-testing	Gallagher *et al*., 2012
45 nursing home residents Romania	Bread fortified with 125 µg vitamin D_3 + 800 mg calcium for 1y	2.29 ± 0.15 Mean ± SD	2.28 ± 0.15	28.8 ± 9.9	126.4 ± 37.3	No apparent adverse effects	Mocanu *et al*., 2009
438 overweight or obese subjects 21-27Y	1000 D_3 500/week Placebo for 1	2.31 ± 0.11 (all subjects)	↑0.00 ± 0.12 ↓0.01 ± 0.11 ↓0.01 ± 0.11	58.7 ± 21.2 56.7 ± 21.2 58.8 ± 21.0	↑79.3 ± 31.2 ↑42.8 ± 22.5 ↓1.6 ± 16.8	Slight increases in systolic blood pressure.	Jorde *et al*., 2010

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
Norway	year	Mean ± SD					
297 post-menopausal women Norway	162.5 D_3 20 µg/day For 1 y	2.36 ± 0.09 2.36 ± 0.07 Mean ± SD	0.02 ± 0.09 0.00 ± 0.10	71 ± 23 71 ± 22	114.7 ± 34.6 18.0 ± 18.9	No difference between groups	Grimnes *et al*., 2012
12 healthy men Sweden	450 D_3 or controls 3 x week for 7 weeks.	2.47 2.50	2.50 2.44 No difference by unpaired t test	38 ± 4 37 ± 2	123 ± 5 (sig. increase) 48 ± 3	No side effects recorded. No significant change in urinary Ca excretion (but became sig when paired t test used. 4.8 and 6.2 mmol/24h in test and 4.2 and 4.5 mmol/24h in controls.	Berlin *et al*., 1986
67 healthy men Omaha, US	0 25, 125 250 vitamin D_3 /day for 20 weeks		Limited data presented but stated that no value rose above the normal range.	70.3	↓11.4 ±4.4 ↑12.0 ± 4.0 ↑91.3 ±9.4 ↑158.4 ± 16.7		Heaney *et al*., 2003
31 patients with corticosteroid induced osteopenia	1125 D_2 2x week for 24 wks + sodium fluoride and calcium phosphate or control for 24 weeks	2.48 ± 0.05 2.50 ± 0.04 Mean ± SD	2.46 ± 0.03 2.44 ± 0.05	4.2 ± 1.2 8.7 ± 2.2	145.3 ± 19.0 7.2 ± 3.5	No hypercalcaemia reported.	Rickers *et al*., 1982 Abstract only (some details taken from EFSA, 2012)
163 patients with spinal crush fracture osteoporosis Denmark	450 D_2 + sodium fluoride and calcium phosphate /day for 5 y	2.50 ± 0.1 Mean ± SD	2.45 ± 0.06			No hypercalcaemia or kidney stones reported.	Hasling *et al*., 1987
116 healthy	25,	2.41	No significant	67 ± 25	↑28.6		Barger-Lux *et*

Participants	Dose (µg) and Duration of vitamin D	Baseline Ca mmol/L	Ca after treatment mmol/L	25 (OH) D before treatment nmol/L	25 (OH)D after treatment nmol/L	Other adverse effects assessed or reported	Reference
men Boston, US	250 or 1250 vitamin D_3 /day for 8 weeks,		changes		↑146.1 ↑643.0		*al*., 1998

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