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

## SACN Review of vitamin D. Adverse effects of high levels: Vitamin D and all-cause mortality

## Introduction

1. Members will recall that the Scientific Advisory Committee on Nutrition (SACN) is in the process of reviewing its recommendations on vitamin D. The COT has been asked to provide advice on high levels of vitamin D intake.

2. A paper providing an overview of vitamin D toxicity (TOX/2012/23) was considered in June 2012; it had been agreed that the 2011 review by the US Institute of Medicine Food and Nutrition Board (IOM, 2011) could be used as a bibliographic source.

3. One of the issues identified by IOM and considered in paper TOX/2012/23 was that of all-cause mortality. When the IOM reviewed vitamin D they identified five cohort studies which focussed on the association between serum 25-hydroxyvitamin D [25(OH)D] levels and all-cause mortality. The studies were designed to explore the hypothesis that increased all-cause mortality was associated with lower serum 25(OH)D levels. These studies were Sambrook *et al*., 2004, 2006; Jia *et al*., 2007; Visser *et al*., 2006; Melamed *et al*., 2008. Semba *et al*., 2009. IOM noted that several of these studies had reported a U or reverse J shape dose-response curve for vitamin D and all-cause mortality.

4. In general, the available studies indicated that low serum 25(OH)D levels (<30 nmol/L) were associated with an increased risk of mortality, thereafter mortality decreased as serum 25(OH)D increased. However, as noted, some of the studies (Jia *et al*., 2007; Visser *et al*., 2006; Melamed *et al*., 2008) suggested a U-shaped response, with a flattening of the dose-response curve and a slight increase in all-cause mortality being observed at the highest serum 25(OH)D levels ($4^{th}$ quartile compared to the $3^{rd}$). The relevant section of TOX/2012/23 is attached at Annex A.

5. On the basis of this information, IOM concluded that serum 25(OH)D levels should not exceed125-150 nmol/L.

6. In their discussion of this issue Members considered that: *"It was noted that the non-monotonic exposure-response relationships which had been observed for some end-points such as all-cause mortality, had a reverse J- shape rather than a U-shape, with higher risk in the lowest category of serum vitamin D levels than in the highest category. The elevation of risk at higher serum levels might reflect confounding (some types of illness leading to increased serum levels and also*

*predisposing to earlier death), and was not necessarily causal".* The complete minutes are attached at Annex B.

## Additional data on vitamin D and all-cause mortality

7. Since this issue was considered, several new studies and meta-analyses have been published and these, as well as others not considered by IOM, have been considered below. In interpreting these studies it should be noted that that normal circulating levels of 25 (OH)D are in the range 25-200 nmol/L (10-40 ng/mL) Jones., 2008). In the UK, data from the National Diet and Nutrition Survey (NDNS) rolling programme up to 2012 (Bates *et al.* 2012) reported that 25(OH)D levels were 46.1 [range 7.07 - 156] and 42.5 [range 7.07 - 110] nmol/L in boys and girls aged 11-18 years (y) respectively and 45.6 [range 7.07 - 123] and 49.6 [range 7.07 - 132] nmol/L in men and women aged 19-64 y respectively.[1]

*Individual studies*

8. In a study by LaCroix *et al*., (2009) 36,282 post-menopausal women aged 51-82 y from the US Women's Health Initiative (WHI) calcium plus vitamin D supplementation trial were given 10 µg vitamin D plus 1000 mg calcium or placebo, daily for a mean of up to 7 y. Exclusion criteria for the study included the use of vitamin D supplements > than 15 µg/day or calcium supplements greater than 1000 mg/day. The hazard ratio (HR) (95%CI) for total mortality was 0.91 (0.83-1.01) with 744 deaths in the treatment group and 807 deaths in the placebo group. The HRs were in the direction of reduced risk but non-significant for stroke and cancer mortality, but near unity for coronary heart disease and other causes of death. HR (95%CI)s for total mortality were 0.89 (0.79-1.01) and 0.95 (0.80-1.12) in the women aged <70 and ≥ 70 respectively. A nested case-control study of baseline 25(OH)D levels in 323 women who had died and 1,962 living controls was also conducted which indicated that the intervention effects did not vary significantly by baseline 25(OH)D level although the odds ratio (OR) was in the direction of benefit for women in the lowest tertile. Compared with women in the highest tertile of serum 25(OH)D (>52.4 nmol/L) there was a significantly increased risk of death for women in the middle (35.4-52.4 nmol/L) and low (<35.4 nmol/L) tertiles. The authors concluded that the results supported the hypothesis that vitamin D and calcium supplementation provided a modest reduction in the rates of total and categories of cause specific mortality but that the results were too imprecise to be definitive. It was further concluded that the study could not distinguish between the effects of vitamin D, calcium or carbonate because the intervention combined all these compounds and it was also unknown whether a higher dose of vitamin D might have produced different results. The serum level of 25(OH)D was not measured post-intervention.

9. Michaëlsson *et al*., (2010) reported data from the Uppsala Longitudinal Study of adult men, a community based study of all men born between 1920 and 1924 and

[1] **NB**: The minimum is 7.07 nmol/L in each case. This is a notional value used when the actual result was below the limit of detection of 10 nmol/L, calculated by dividing the limit of detection by the square root of 2.

living in the Uppsala area of Sweden. The study began in 1970, but the data in the present analysis was based on the measurements made in the third examination cycle of the cohort, when the participants had a mean age 71 y, and of these individuals,1194 had baseline measures of serum 25(OH)D; during the follow up period (median 12.7 y) 584 (49%) of the participants died. Cox proportional hazards models were used to estimate HRs and 95%CIs. To allow for a non-linear association of vitamin D with mortality, plasma 25(OH)D was modelled as a continuous variable with linear and quadratic terms. Additional analysis for a non-linear trend was conducted by using a restricted cubic-spline Cox regression analysis. The results were presented as a smooth plot of CIs with 4 "knots" at the recommended 5$^{th}$, 35$^{th}$. 65$^{th}$ and 95$^{th}$ percentiles with a concentration of 80 nmol/L used as a reference, being a suggested optimal level (see Table 1). There was a U shaped association between vitamin D concentration and total mortality. An approximately 50% higher total mortality was observed among men in the lowest 10% and highest 5% serum 25(OH)D concentrations compared with intermediate concentrations HR (95%CI) 1.61 (1.15-2.22) (*P* = 0.0009 for plasma vitamin D as a quadratic term). Cancer mortality was also higher at low and high plasma concentrations 2.20 (1.44-3.38) and at a high concentration 2.64(1.46-4.78) (*P* = 0.0004 for plasma vitamin D as a quadratic term). For cardiovascular death only, low (but not higher) 25(OH)D concentrations indicated higher risk (1.89 (1.21-2.96) and 1.33 (0.69-2.54) respectively) (non-significant quadratic term, *P*= 0.52). The authors noted experimental data, including animal and *in vitro* studies which suggest high vitamin D could accelerate aging and increase the risk of cancer.

Table 1. HR (95%CIs) of mortality by percentile categories of plasma 25-hydroxyvitamin D (Michaëlsson *et al*., 2010)

| | <5$^{th}$ percentile | <10$^{th}$ percentile | 10-90$^{th}$ percentile | >90$^{th}$ percentile | >95$^{th}$ percentile |
|---|---|---|---|---|---|
| 25(OH)D nmol/L | <39 | <46 | 46-93 | >93 | >98 |
| Overall mortality | | | | | |
| No of deaths | 37 | 76 | 444 | 64 | 39 |
| HR (95%CI) | 1.44 (1.02-2.02) | 1.65 (1.29-2.11) | 1.0 referent | 1.27 (0.98-1.66) | 1.61 (1.15-2.22) |
| Non cancer mortality | | | | | |
| No of deaths | 21 | 47 | 269 | 45 | 27 |
| HR (95%CI) | 1.40 (0.89-2.21) | 1.63 (1.21-2.24) | 1.0 referent | 1.18 (0.84-1.65) | 1.47 (0.96-2.24) |
| Cardiovascular mortality | | | | | |
| No of deaths | 9 | 24 | 135 | 18 | 10 |
| HR (95%CI) | 1.26 (0.62-2.54) | 1.89 (1.21-2.96) | 1.0 referent | 1.15 (0.70-1.90) | 1.33 (0.69-2.54) |
| Cancer mortality | | | | | |
| No of deaths | 12 | 27 | 118 | 19 | 13 |
| HR (95%CI) | 2.41 (1.41-4.12) | 2.20 (1.44-3.38) | 1.0 referent | 1.54 (0.94-2.54) | 2.64 (1.46-4.78) |

The model was adjusted for age, weight, height, calcium intake, alcohol intake, season of blood draw, social class, smoking status and leisure physical activity.

10. The association between plasma 25(OH)D levels and cause-specific mortality was examined in the Whitehall study, a prospective study of older UK men (Tomson *et al* 2012). This study consisted of 19,019 male civil servants who were working in London at the time of recruitment in 1967-70. A re-survey was conducted in 1995 of the surviving 8448 participants, with complete information and blood results being available from 5409 of them. In a 13 year follow up period, 1358 of the 5409 men (mean baseline age 77 y), died from vascular and 1857 from non-vascular causes. The median season-adjusted baseline 25(OH)D concentration was 56 (interquartile range 45-67) nmol/L. After adjustment for age and seasonality, higher 25(OH)D concentrations were inversely and approximately linearly associated with vascular and non-vascular mortality throughout the range 40-90 nmol/L. Some attenuation of risk for non-vascular mortality was apparent at concentrations of 25(OH)D > 80 nmol/L. Given age, a doubling of 25(OH)D concentration was associated with an average 34% lower relative risk (RR) of vascular mortality (RR (95%CI) 0.66 (0.58-0.75) and 36% lower risk of non-vascular mortality 0.64 (0.58-0.72); after adjustment for prior disease this was reduced to 20% lower risk of vascular mortality 0.80 (0.70-0.91) and 23% lower risk of non-vascular mortality 0.77 (0.69-0.86). The findings of Zitterman *et al*., 2012 were noted (an increased risk >87.5 nmol/L) but there were too few individuals with 25(OH)D levels above this to confirm or refute this. It was noted that despite the strong association, causality remained uncertain and that large scale trials would be necessary to establish clinical relevance.

11. In a study of 1801 patients with metabolic syndrome from the LURIC cohort (Ludwigshafen Risk and Cardiovascular Health) in South West Germany, optimal 25(OH)D levels (≥ 70 nmol/L) were associated with substantially reduced levels of all-cause and cardiovascular mortality compared to those with severely deficient (<25 nmol/L) levels (HR: 95% CI, 025; 0.13-0.46) (Thomas *et al*., 2012). The participant groups had a mean age of 61.7 to 66.0 y depending on the group, and the numbers of women also varied between groups. In a median follow up period of 7.7 y, 462 deaths occurred, of which 57.8% were cardiovascular in origin. It was reported that the majority of the subjects had sub-optimal levels of 25(OH)D. Therefore it seems unlikely that this study could detect any adverse effects at higher levels of intake.

12. A total of 9,146 individuals from the Monica10 (1993-94) and Inter99 (1999-2001) studies were included in a study by Skaaby *et al*., (2012) which compared vitamin D status and mortality in a Danish population. The participants had a mean age of 49.8 y (range 30-60y) and were evenly distributed between the sexes. This is therefore a much younger population than considered in other studies. Vitamin D levels were divided into quartiles before the two studies were merged, to account for the different methods of measuring vitamin D and the differing storage times. Baseline median vitamin D levels were 61 nmol/L (interquartile range 44.7-80.9 nmol/L) and 48 nmol/L (interquartile range 32.65.0 nmol/L) in the Monica10 and Inter99 studies respectively. A total of 832 deaths occurred in a median follow up period of 10.3 years. Multivariable Cox regression analysis with age as underlying time axis and vitamin D quartiles showed significant association between vitamin D status and death caused by diseases of the respiratory system, the digestive system

and endocrine, nutritional and metabolic diseases with HRs of 0.26 ($P_{trend}$ = 0.0042), 0.28 ($P_{trend}$ = 0.0040) and 0.21 ($P_{trend}$ = 0.0035) respectively for the fourth vitamin D quartile compared to the first. Non-significantly lower HRs were found for death caused by mental and behavioural diseases of the nervous system, but no association between vitamin D status and death caused by neoplasms or diseases of the circulatory system (in contrast to a number of other reports).

13. In a study by Dror *et al.,* 2013[2] data were extracted for 1,282,822 Clalit Health service members (Israel) aged > 45 y between July 2007 and December 2011, records of mortality or acute coronary syndrome were extracted in the follow up period. Kaplan Meier analysis calculated time to episode and Cox regression models adjusted HRs for 25(OH)D concentrations <24.96, 25.2- 49.92, 50.17- 89.86 and > 90.11 nmol/L. The outcome measure was acute coronary syndrome subsuming all-cause mortality. During the study period, 422 Clalit Health Service Members were tested for 25(OH)D, of these 12,280 died of any cause (905 with acute coronary syndrome) and 3,933 were diagnosed with acute coronary syndrome. Compared to those with 25(OH)D of 50.17- 89.86 nmol/L, the adjusted HRs were 1.88 [CI:1.80-1.96], 1.25 [CI:1.21-1.30] and 1.13 [CI:1.04-1.22] for 24.96, 25.2- 49.92 and > 90.11 nmol/L respectively (p< 0.05) (see Table 2). It was noted that the study cohort contained only 30% of the population (those tested for vitamin D) and the sample size of the > 90.11 nmol/L group prevented further analysis.

Table 2. HR (95%CIs) of mortality by percentile categories of plasma 25-hydroxyvitamin D (Dror *et al*., 2013)

| 25 (OH)D nmol/L. | Deaths | HR (95%CI) |
|---|---|---|
| <24.96 | | 1.88 (1.80-1.96) |
| 25.2- 49.92 | | 1.25 (1.21-1.30) |
| 50.17- 89.86 | | 1.00 referent |
| 90.11 | | 1.131.04-1.22) |

14. Commenting on this study, Eisman (2013) considered that this was really a reverse J shaped relationship, and that there was no apparent dose-response at the top end of the concentration range, although this might be due to low numbers of individuals. Whilst it was reasonable to suggest caution regarding vitamin D supplementation, this could be overstating matters in the absence of data from randomised controlled trials. It was also pointed out that a strength of the study was that it was a genuine sample of the population, but that the measured individuals represented approximately 33% of the total health fund membership over the age of 45 y who might be expected to have lower 25(OH)D levels eg current smokers and the overweight/obese. The follow up period was also likely to be short for some participants.

15. Concentrations of serum 25(OH)D were measured in 9578 baseline patients and 5469 follow up participants of the ESTHER study (a German population based

[2] Original not yet obtained.

cohort study) (Schottker *et al*., 2013a). Deaths were recorded during 9.5 years of follow up. Restricted cubic splines were used to assess dose-response relationships and Cox regression with time-dependent variables was used to estimate hazard ratios. During the follow up period, 1083 study participants died (350 of cardiovascular disease [CVD], 433 of cancer and 55 of respiratory disease). The overall mortality [HR (95%CI)] of subjects with vitamin D deficiency (25(OH)D < 30 nmol/L) or vitamin D insufficiency (25(OH)D 30-50 nmol/L) was significantly increased (1.71 (1.43-2.01) and 1.17(1.02-1.35) respectively compared to subjects with sufficient vitamin D (25(OH)D (> 50 nmol/L). Vitamin D deficiency was also associated with increased cardiovascular mortality, cancer mortality and respiratory disease mortality. However the association between 25(OH)D concentration and all-cause mortality proved to be a non-linear inverse association with risk that started to increase at serum 25(OH)D concentrations < 75 nmol/L. Two additional sensitivity analyses were conducted, one using an additional group with serum 25(OH)D levels of 50-75 nmol/L and using the group with serum concentrations >75 nmol/L as the reference group, and the second using quintiles. The data were not reported but it was stated that the results were very similar. There appeared to be no indication of increasing risk at higher serum concentrations, though it was noted that the sample size of subjects with 25(OH)D concentrations > 112.5 nmol/L was small.

*Meta-analyses*

16. In a meta-analysis of 18 randomised controlled trials (RCTs) including 57,311 participants (Autier and Gandini, 2007) a total of 4777 deaths occurred during a mean of 5.7 y. The daily dose of vitamin D supplementation ranged from 7.5 to 50 µg (mean 13.2 µg). In 9 trials there was a 1.4-5.2 fold difference in serum 25(OH)D concentrations between the intervention and control groups. The summary relative risk for mortality was 0.95 (95%CI; 0.87-0.99) indicating that there was a reduced risk of mortality in the intervention group compared to the controls. There was no indication of heterogeneity or of publication bias. The majority of the trials also supplemented participants with calcium, but the summary relative risk did not change according to the addition of calcium supplements in the intervention. The trials included in the analysis were Chapuy *et al*., 1992, Lips *et al*., 1996, Baeksgaard *et al*., 1998, Komulainen *et al*., 1999, Krieg *et al*., 1999, Chapuy *et al*., 2002 Meyer *et al*., 2002, Trivedi *et al*., 2003, Latham *et al*., 2003, Harwood *et al*., 2004, Avenell *et al*. 2004, Meier *et al*., 2004, Brazier *et al*., 2005, Porthouse *et al*., 2005, RECORD trial 2005, Flicker *et al*., 2004, Schleithoff *et al*., 2006, Jackson *et al*., 2006 and Wactawski-Wende *et al*., 2006[3]. The majority of the trials were designed to assess bone health and related endpoints, but also assessed the survival of patients with congestive heart failure and colorectal cancer incidence. Sub-group analysis did not affect the conclusions of the study. A summary relative risk (95% CI) of 0.93 (0.85-1.03) was calculated for the 12 studies using ≥ 20 µg vitamin D/day and 0.92 (0.25-1.03) for the 6 studies using < 20 µg vitamin D. The authors concluded that the relationship between baseline vitamin D status, dose of vitamin D supplements, and total mortality rates remained to be investigated and that population based, placebo-

[3] A number of these studies have been considered in earlier COT papers on vitamin D but have not been discussed here.

controlled randomised trials with total mortality as the endpoint were needed to confirm their findings.

17. A meta-analysis of 10 studies conducted by Pilz *et al*., (2011) (including 6853 patients with chronic kidney disease (CKD) overall) reported that the RR for mortality was 0.86 (0.82-0.91) for each 25 nmol/L increase in 25(OH)D. Vitamin D deficiency is common in CKD patients so it is unclear whether any of the participants would have had 25(OH)D levels in the range that may be of concern, the highest category being >74.7 nmol/L.

18. In a Cochrane review of vitamin D supplementation for the prevention of mortality (Bjelakovic *et al*., 2011) it was reported that overall, vitamin D decreased mortality RR (95%CI) 0.97 (0.94-1.01). However, when analysed separately only vitamin D3 decreased mortality significantly (RR, 0.94(0.91-0.98), $I^2$ = 0%; 74,789 participants, 32 trials) whereas the other forms did not. The majority of the trials were conducted in elderly women who were in institutional or dependent care. This beneficial effect was apparent at doses lower than 20 µg vitamin D3. A U shaped response was noted in some studies. The review observed that vitamin D2 could increase mortality in trials with a high risk of bias, as well as in the vitamin D insufficient participants (RR, 1.20(1.05-1.37), *P*= 0.008 $I^2$ = 0%) possibly due to a random error. In this analysis there were 2129 participants in the treatment group and 2284 in the controls with 378 and 340 events respectively.

19. Zitterman *et al*., 2012 conducted a meta-analysis of 14 prospective cohort studies; these were Visser *et al*., 2006; Jia *et al*., 2007; Melamed *et al*., 2008; Kuroda *et al*., 2009; Pilz *et al*., 2009; Kilkkinen *et al*., 2009; Semba *et al*., 2009; Semba *et al*., 2010; Szule *et al*., 2010; Bolland *et al*., 2010; Hutchinson *et al*., 2010; Anderson *et al*., 2010; Cawthorn *et al*., 2010; Michaëlsson *et al*., 2010 and included 5562 deaths out of 62,458 individuals in a non-parametric model. Log-transformed RRs and CIs adjusted for the maximal number of confounding variables were applied. In this, the summary estimate for highest versus lowest categories of 25(OH)D showed a significant mortality risk reduction of 0.71 (95%CI 0.50-0.91, $I^2$ =58%). In a parametric model, based on 11 studies (Visser *et al*., 2006; Jia *et al*., 2007; Melamed *et al*., 2008; Kilkkinen *et al*., 2009; Semba *et al*., 2009; Semba *et al*., 2010; Szule *et al*., 2010; 2010; Hutchinson *et al*., 2010; Anderson *et al*., 2010; Cawthorn *et al*., 2010; Michaëlsson *et al*., 2010) and including 59,231 individuals the lowest quantile was used as the reference category. In this parametric model, the estimated summary RRs (95%CI) of mortality were 0.86 (0.82-0.91), 0.77 (0.70-0.84 and 0.69 (0.60-0.78) for individuals with an increase of 12.5, 25 and 50 nmol/L serum 25(OH)D from a median reference category of 27.5 nmol/L. There was no significant decrease in mortality when an increase of 87.5 nmol/L above the reference category occurred. The authors concluded that the data suggested a nonlinear decrease in mortality risk as circulating 25(OH)D increased, with optimal concentrations being 75-87.5 nmol/L. It was noted that in the meta-analysis, it was not possible to assess the association between 25(OH)D concentrations and mortality risk above a concentration of 112.5 nmol/L and the data did not exclude the possibility of the risk rising above this level, with some of the included cohort studies (Melamed *et al*., 2008; Michaëlsson *et al*., 2010) reporting an increased risk of mortality at concentrations > 97.5 nmol/L. Commenting on this meta-analysis,

Schottker *et al*., 2013a stated that the non-significant increase at 25(OH)D concentrations> 112.5 nmol/L was largely driven by the results of the Michaëlsson *et al*., (2010) study.

20. Tomson *et al*., 2012 conducted a meta-analysis of 12 prospective studies with 4632 vascular deaths (Cawthorn *et al*., 2010; Hutchinson *et al*., 2010; Jassal *et al*., 2010; Kestenbaum *et al*., 2011; Kilkkinen *et al*., 2009; Melamed *et al*., 2008; Michaëlsson *et al*., 2010; Pilz *et al*., 2009; Semba *et al*., 2010; Virtanen *et al*., 2010; Wang *et al* , 2008 and the Whitehall study (see paragraph 10 above) and 18 studies (Anderson *et al*., 2010; Cawthorn *et al*., 2010; Ensrud *et al*., 2010; Ford *et al*., 2011; Hutchinson *et al*., 2010; Jia *et al*., 2007; Kestenbaum *et al*., 2011; Kitamura *et al*., 2010; Kuroda *et al*., 2009; Melamed *et al*., 2008; Michaëlsson *et al*., 2010; Pilz *et al*., 2009; Semba *et al*., 2010; Semba *et al*., 2009; Szule *et al*., 2010; Virtanen *et al*., 2010; Visser *et al*., 2006; Wang *et al* , 2008 and the Whitehall study) with 11374 deaths from all causes. Individuals in the top versus bottom quartile had a 21% (95%CI 13-28%) lower vascular risk and 23% (95%CI 24-32%) lower all-cause mortality. Observed RRs varied inversely with the amount of statistical information provided by each study (i.e. study size) with more extreme estimates being seen among smaller studies for both vascular and non-vascular mortality.

21. The association between vitamin D status (serum 25(OH)D) and cardiovascular disease was examined in a meta-analysis of 19 independent studies which included 6123 cases and 65,994 participants (Wang *et al*., 2012). The studies used were Marniemi *et al*., 2005; Wang *et al,* 2008;Giovanucci *et al*., 2008; Pilz *et al*., 2008; Melamed *et al*., 2008; Pilz *et al*., 2009; Kilkkinen *et al*., 2009; Bolland *et al*., 2010; Semba *et al*., 2009; Anderson *et al*., 2010; Cawthorn *et al*., 2010; Michaëlsson *et al*., 2010; Hutchinson *et al*., 2010; Jassal *et al*., 2010; Hosseinpanah *et al*., 2011; Eaton *et al*., 2011 Kestenbaum et al., 2011. In a comparison of the highest and lowest 25(OH)D categories, the pooled RR (95%CI) was 1.53 (1.30-1.77) for total CVD 1.42 (1.19-1.71) for CVD mortality, 1.38 (1.21-1.57) for coronary heart disease and 1.64 (1.27-2.10) for stroke. The associations remained strong and significant when analyses were limited to studies that excluded participants with baseline CVD and were better controlled for season and confounding. Fractional polynomial spline regression analysis was used to assess the linearity of the dose-response association between continuous 25(OH)D and risk. The CVD risk increased monotonically across decreasing 25(OH)D below 60 nmol/L with a RR (95%CI) of 1.03(1.00-1.06) per 25 nmol/L decrement in 25(OH)D. There was no clear decrease or increase in CVD risk for 25(OH)D > 60 nmol/L based on the few data points available. It was concluded that further research was needed to clarify the association between 25(OH)D and CVD risk at 25(OH)D concentrations higher than 60 nmol/L.

22. Schöttker *et al*., (2013b) conducted a meta-analysis of 12 original studies (Bates *et al*., 2011; Cawthorn *et al*., 2010; Hutchinson *et al*., 2010; Jia *et al*., 2007; Kestenbaum *et al*., 2011; Melamed *et al*., 2008; Michaëlsson *et al*., 2010; Pilz *et al*., 2009; Semba *et al*., 2010; Szulc *et al*., 2009; Virtanen *et al*., 2010; Visser *et al*., 2006), comprising 32,141 participants (largely elderly) with measured 25(OH)D of whom 6921 died during follow up. Reported HRs for 25(OH)D categories were

recalculated using comprehensive trend estimation from summarized dose-response data and pooled in a random effects model meta-analysis. An inverse association between 25(OH)D levels and all-cause mortality was found in all but two studies that was statistically significant in several of the individual studies. In meta-analysis, 25 (OH)D levels were significantly associated with all-cause mortality with a pooled HR; 95%CI of 0.92; 0.89-0.95 for a 20 nmol/L increase in 25(OH)D levels. This paper has been attached at annex C as it provides a helpful visual summary of the data.

*Other studies*

23. A Mendelian randomisation study was performed by Trummer *et al*., (2013) which analysed whether 3 common single nucleotide polymorphisms (SNPs) associated with 25(OH)D concentrations were causal. Genotypes of SNPs in the group-specific component gene (*GC*, rs2282679), 7-dehydrocholesterol reductase gene (*DHCR7*, rs12785878) and cytochrome P450 IIIR-1 gene (*CYP2R1*, rs10741657) were determined in a prospective cohort study of 3316 male and female participants (mean age 62.6 y) scheduled for coronary angiography. In a linear regression model adjusting for month of blood sampling, age and sex, vitamin D concentrations were predicted by *GC* genotype (*P* <0.001), *CYP2R1* genotype (*P* <0.068) and *DHCR7* genotype (*P* <0.001) with a coefficient of determination $r^2$ of 0.175. During a median follow up time of 9.9 y, 955 persons died including 619 deaths from cardiovascular cause. In a multivariate Cox regression adjusted for classical risk factors, *GC*, *CYP2R1* and *DHCR7* genotypes were not associated with all-cause mortality, cardiovascular mortality or non-cardiovascular mortality. The authors concluded that low 25(OH)D concentrations were associated with, but unlikely to causal for higher mortality rates.

*Comments on the "U shaped curve"*

24. Grant (2009) reviewed the reports of a U shaped dose-response relationship for vitamin D in several areas including that of all-cause mortality. It was noted that the Melamed *et al*. (2008) study was used to support the concept of such a relationship, but for both quintile and quartile analysis, only the lowest quantile was statistically significantly different from 1.0 therefore it was "a stretch" to state that it supported a U shaped relationship; it would be more appropriate to do a meta-analysis than to single out a particular study. Overlaying the data from 3 other comparable studies (Dobnig *et al*., 2008; Ginde *et al*., 2009; Semba *et al*., 2010) and adjusting the HRs, the power law and logarithmic fits to the data decrease monotonically out to 107 nmol/L, with a value from Dobnig *et al*., 2008 cancelling out the equivalent from Melamed *et al*., 2008.

## Summary and discussion

25. In its review of vitamin D, the US IOM identified a U or reverse J shaped dose-response relationship for vitamin D and recommended that blood 25(OH)D levels should not exceed 125-150 nmol/L. A number of additional studies have been considered in this paper, both individual studies and meta-analyses. In general, an increase in the risk of all-cause mortality is not found, though as the majority of

studies compare highest and lowest quantiles rather than comparing both against a mid, or optimal range they may not be relevant and any increased risk at low vitamin D concentrations might mask effects at higher concentrations. However, Michaëlsson *et al*., 2010 found a significant increase in the risk of overall and cancer mortality. In addition, Dror *et al*., 2013 reported a slight increase in mortality at the highest serum 25(OH)D levels. The results of the meta-analyses are also mixed, with some reporting no elevation of risk at high 25(OH)D concentrations, but others a small increase, though this may be driven by particular studies.

26. The reasons for the differing results are uncertain. The numbers of participants with high 25(OH)D concentrations are likely to be small, but possibly dependent on latitude, again reducing the likelihood of detecting any effect. Many supplementation studies do not provide baseline or post-treatment 25(OH)D levels so it is unclear how actual serum levels could affect the findings. The prospective cohort studies are largely population-based so should be reasonably comparable with respect to gender and health status but there could be differences in ages and ethnicity depending on location and date of recruitment.

27. The meta-analyses use slightly differing studies, though the majority are common to all of the analyses (the individual studies involved have not been considered in detail due to time constraints). Finally it has been argued that the U shaped curve may be a statistical artefact.

**Questions for the Committee**

28. The Committee are asked:

i. Whether the U (or reverse J) shaped curve is a real effect?

ii. Can any conclusions be drawn about high levels of serum vitamin D and mortality for the general population?

iii. Can any conclusions be drawn about high levels of serum vitamin D and mortality for specific population groups?

iv. Any other comments they may have.

**Secretariat**
**August, 2013.**

Glossary

BMI: Body Mass Index
CI: Confidence Intervals
CKD: chronic kidney disease
CVD: cardiovascular disease
CYP: cytochrome P450
HR: Hazard Ratio
25(OH)D: 25-hydroxyvitamin D
IOM: Institute of Medicine Food and Nutrition Board
OR: Odds Ratio
RCT: randomised controlled trial
RR: relative risk
SACN: Scientific Advisory Committee on Nutrition
SNP: Single Nucleotide Polymorphism.
US: United States of America

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

### SACN Review of vitamin D. Adverse effects of high levels. All cause mortality

68. Five cohort studies were identified by IOM (2011) which focussed on the association between serum 25(OH)D levels and all-cause mortality. The studies were designed to explore the hypothesis that increased all-cause mortality was associated with lower serum 25(OH)D levels. In general the available studies indicated that low serum 25(OH)D levels (<30 nmol/L(75 ng/ml)) were associated with an increased risk of mortality, thereafter mortality decreased as serum 25(OH)D increased. However some of the studies suggested a U- shaped response, with a flattening of the dose-response curve and a slight increase in all cause mortality being observed at the highest serum 25(OH)D levels. In interpreting these studies it should be noted that that normal circulating levels of 25 (OH)D are in the range 25-200 nmol/L (10-50 ng/ml) Jones., 2008).

69. Jia *et al*., (2007) reported a statistically significant trend between increasing serum 25(OH)D levels and lowered odds ratios for all cause mortality (*p*= 0.03) in a population of community dwelling men (n= 208) and women (n=191) aged over 75y living in Scotland. The participants had been recruited for a cross-sectional study in 1999-2000 when questionnaires on lifestyle were completed and blood samples taken; mortality was assessed at the end of 2005 (median follow up 69.2 months). Hazard ratios were estimated using Cox proportional hazard models for quintiles 1-4 referent to quintile 5. The IOM (2011) considered that a U or reverse J shaped relationship was apparent with the lowest mortality at serum levels below 50 nmol/L(20 ng/ml); this was not discussed by the authors). See Fig 1 below taken from IOM (2011). The Hazard ratios for the different quintiles are given in Table 4 below:

Table 4.Hazard Ratios of death referent to the highest quintile of serum 25(OH)D from Jia *et al*., (2007)

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

*Model 3, adjusted for age, sex, taking ≥5 medicines, self-perceived health status and pre-existing heart disease and/or diabetes,
‡Model 4. Model 3 + sunlight exposure,
†Model 5, Model 3 + use of supplement s containing vitamin D,
§Model 6. Model 3 + variables in models 4 and 5 .
In ng/mL the quintiles are: 2.4-9.2, 9.24-12, 12.04-14.8, 14.84-18.8, 18.84-32.8 in men and, 2.8-7.6, 7.64-9.24, 9.64-12.08, 12.12- 15.6, 15.64-32.8 in women.

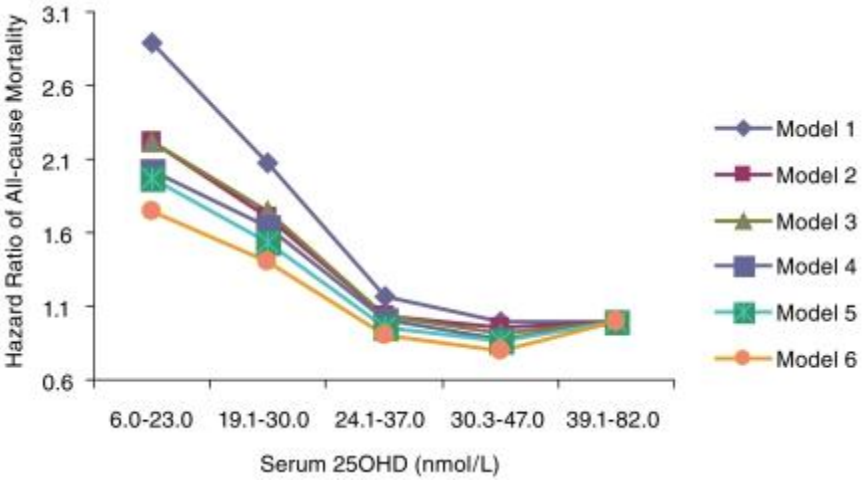


**Fig 1. Hazard ratios of risk of death according to baseline serum 25OHD level (subjects with serum 25OHD levels 39.1–82.0 nmol/L are the referent category)**
NOTE: Model 1 is adjusted for age and gender; model 2 is adjusted for model 1 and taking five or more kinds of medicine and self-perceived health status; model 3 is adjusted for model 2 and having heart problem and/or diabetes at baseline; model 4 is adjusted for model 3 and sunlight exposure (i.e., season of blood sampling, sunbathing, and outdoor physical activity); model 5 is adjusted for model 3 and use of a supplement containing vitamin D; model 6 is adjusted for model 3 and variables in models 4 and 5.

70. A similar pattern was reported by Visser *et al*., (2006) with reduced mortality at higher than deficiency levels of 25(OH)D but an increase in the highest quartile of blood levels (≥75 nmol/L(30ng/ml)). This study was designed to ascertain whether lower serum 25(OH)D levels increased the risk of future nursing home admission and early death. 1260 independent community-dwelling persons aged 65 y or greater (who were participating in the Longitudinal Aging Study Amsterdam) were included; serum vitamin D levels were measured at the first follow up appointment, which was taken as baseline for this part of the study. When stratified by level and adjusted for age, sex and education, low serum 25(OH)D was associated with increased all-cause mortality, however, following further adjustment for health and lifestyle factors and for frailty factors, the relationship was no longer statistically significant.

Table 5.Hazard Ratios of death referent to the highest quartile of serum 25(OH)D from Visser *et al*., (2004)

| | **Model 1*** | **Model 2[§]** | **Model 3[†]** | **Model 4[‡]** |
|---|---|---|---|---|
| | HR (95%CI) | HR (95%CI) | HR (95%CI) | HR (95%CI) |
| **25 OHD nmol/L** | | | | |
| **<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 |

* Model 1 is adjusted for gender, age, and education;
[§] model 2 is adjusted for model 1 + chronic disease, serum creatinine concentration, cognitive status, and depressive symptoms;
[†] model 3 is adjusted for model 2 + lifestyle variables including BMI, smoking status, alcohol consumption, and physical activity;
[‡] model 4 is adjusted for model 3 + frailty indicators: mobility performance, low serum albumin concentration, and low serum total cholesterol concentration.
In ng/mL the quintiles are <10, 10-19.96, 20-29.8, ≥ 30.

The IOM considered that the data suggested a potential U shape since the highest category of serum 25(OH)D levels had a higher association with mortality compared to the third highest; See Fig 2 below taken from IOM (2011). There was no difference in the associations for men and women.

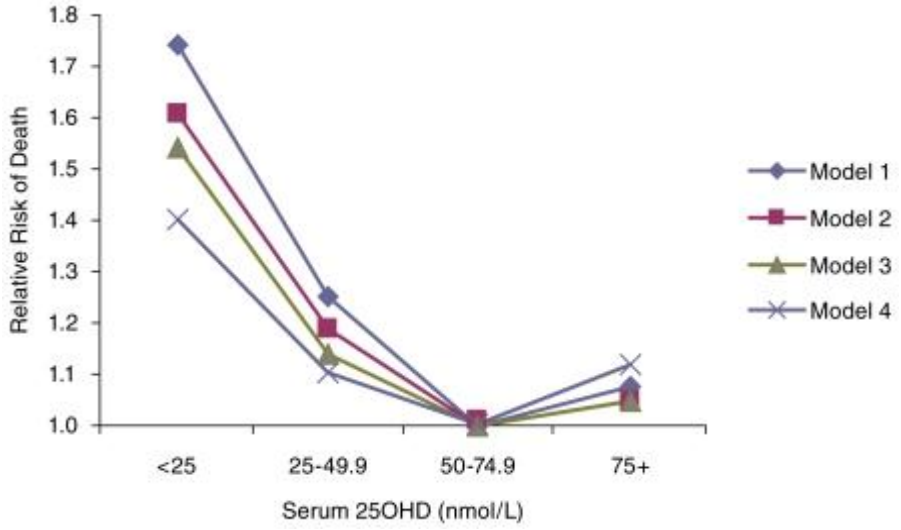


**FIGURE 2. Risk of death in elderly people according to baseline serum 25OHD level in the Longitudinal Aging Study (subjects with serum 25OHD levels of 50.0–74.9 nmol/L are the referent category)**
NOTE: Model 1 is adjusted for gender, age, and education; model 2 is adjusted for model 1 and for chronic disease, serum creatinine concentration, cognitive status, and depressive symptoms; model 3 is adjusted for model 2 and for lifestyle variables including body mass index, smoking status, alcohol consumption, and physical activity; model 4 is adjusted for model 3 and for frailty indicators: mobility performance, low serum albumin concentration, and low serum total cholesterol concentration.

71. Similar findings were reported by Melamed *et al*., 2008. In this observational study, the association between low 25(OH)D levels with all-cause, cancer and cardiovascular disease mortality was tested in 13,331 representative adults ≥ 20y using NHANES III linked mortality data. Serum 25(OH)D levels were collected between 1988-1994, and the participants were then passively followed until 2000. In multivariable analyses adjusted for baseline demographics, season, traditional, vitamin D supplement use and novel CVD risk factors, the lowest 25(OH)D quartile compared to the highest quartile was associated with a 26% increased rate of all cause mortality; see Table 5 below and Fig 3 taken from IOM (2011).

Table 6. Hazard Ratios of death referent to the highest quartile of serum 25(OH)D from Melamed *et al*., (2008)

| | **Unadjusted** | **Limited*** | **Fully‡** | **Fully (no diabetes or hypertension)** |
|---|---|---|---|---|
| | HR (95%CI) | HR (95%CI) | HR (95%CI) | HR (95%CI) |
| **25 OHD ng/mL** | | | | |
| **<17.8** | 1.78 (1.44, 2.21) | 1.52 (1.31, 1.77) | 1.26 (1.08, 1.46) | 1.28 (1.11, 1.48) |
| **17.8-24.4** | 1.49 (1.24, 1.78) | 1.11 (0.95, 1.31) | 1.06 (0.89, 1.24) | 1.06 (0.9,1.26) |
| **24.4-32.1** | 1.14 (0.94, 1.39) | 0.92 (0.78, 1.08) | 0.93 (0.79, 1.10) | 0.94 (0.80, 1.12) |
| **≥ 32.1** | 1.0 | 1.0 | 1.0 | 1.0 |

*Adjusted for age, sex, race, season,

‡ Fully Adjusted Model includes age, sex, race, season, hypertension, history of prior CVD, 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 SES.

Serum 25(OH)D was reported in ng/mL; the equivalent quintiles are <44.5, 44.5-61, 61-80.1 and > 80.1 mmol/L

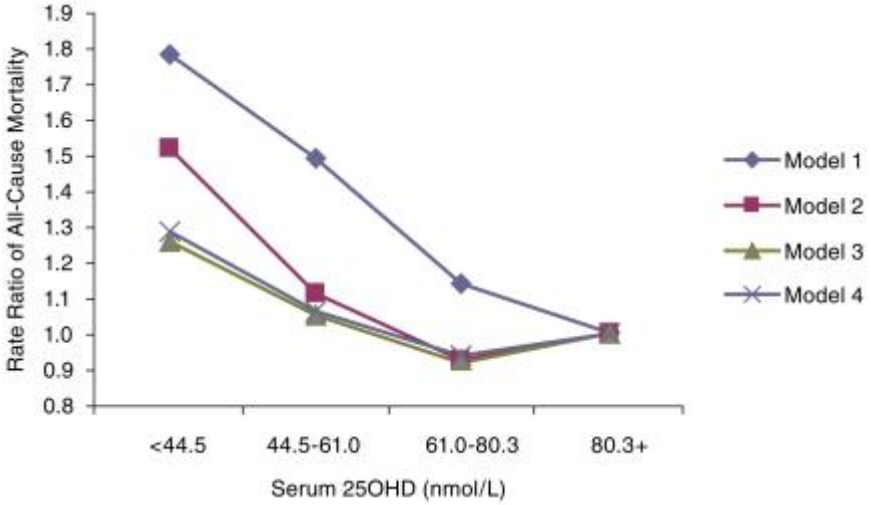


**FIGURE 3. Rate ratios of all-cause mortality by serum 25OHD level in NHANES III (subjects with serum 25OHD levels above 80.3 nmol/L are the referent category)**

NOTE: Model 1 is unadjusted; model 2 is adjusted for age, gender, race, and season; model 3 is adjusted for age, gender, race, season, hypertension, history of prior cardiovascular disease, diabetes, smoking, high-density lipoprotein cholesterol, total cholesterol, use of cholesterol medications, estimated glomerular filtration rate categories, serum albumin, log (albumin-creatinine ratio), log (C-reactive protein), body mass index, physical activity level, vitamin D supplementation, and low socioeconomic status; model 3 is adjusted for age, gender, race, season, cigarette use, body mass index, log (C-reactive protein), serum albumin, physical activity level, vitamin D supplementation, and low socioeconomic status.

However, as with the Visser *et al* (2006) study, the highest quartile of serum 25(OH)D levels had an increased risk of all cause mortality compared to the third highest quartile. The association of all cause mortality was more pronounced in women. In a sub-group analysis, the association between serum 25(OH)D levels and all cause mortality differed in men and women.

Table 7. Hazard Ratios in men and women of death referent to the highest quartile of serum 25(OH)D from Melamed *et al*., (2008)

| | **Men** | **Women** |
|---|---|---|
| | HR (95%CI) | HR (95%CI) |
| **25 OHD ng/mL** | | |
| **<17.8** | 1.04 (0.83-1.30) | 1.51 (1.15, 1.98) |
| **17.8-24.4** | 0.94 (0.75, 1.19) | 1.27 (0.97, 1.66) |
| **24.4-32.1** | 0.82 (0.64-1.05) | 1.16 (0.87, 1.55) |
| **≥ 32.1** | 1.0 | 1.0 |
| ***P*** interaction 0.06 | | |

The quintiles are <44.5, 44.5-61, 61-80.1 and > 80.1 mmol/L

72. However, in a study by Sambrook *et al*., (2005), it was reported that baseline serum 25(OH)D was a significant predictor of time to death in 842 older people

resident in nursing homes in Australia, with higher serum 25(OH)D levels being protective; HR, 0.99; 95%CI 0.982-0.998). The data were analysed by univariate and multivariate models after correcting for age and gender. In analyses which corrected for health status, nutritional status and renal function, 25(OH)D was no longer associated with mortality. The 25(OH)D levels by survivor status were 27.9 nmol/L (11 ng/ml) in the living subjects and 24.9 mmol/L (10 ng/ml) in those that then died. ($p$ =0.006).

73. In a prospective study of NHANES III data (1988-1994), Ginde *et al*., (2009) investigated all cause mortality in 3408 adults aged ≥ 65 y; the median follow up was 7.3 y. Analysis of the fully adjusted data suggested there was an inverse relationship between all cause mortality (n = 1493) and serum 25(OH)D levels. In the fully adjusted model ( age, sex, race/ethnicity, poverty: income ratio, region, BMI, physical activity, smoking, asthma, COPD, renal function, hypertension, diabetes, hyperlipidaemia, MI, stroke and cancer) the HRs (95%CI were:

Table 8. Hazard Ratios of death referent to the highest quintile of serum 25(OH)D from Ginde *et al*., (2009)

| Serum 25 OHD nmol/L (ng/ml) | HR (95% CI) |
|---|---|
| < 25 (10) | 1.83 (1.14-2.94) |
| 25-49.9 (10-20) | 1.47 (1.09-1.97) |
| 50-74.9 (20-30) | 1.21 (0.92-1.59) |
| 75- 99.9 (30-40) | 1.15 (0.86-1.53) |
| 100 (40) | 1.00 (ref) |

74. Semba *et al*., 2009 reported that in a study of 714 community dwelling women in the US, aged 70-79, low serum vitamin D levels were associated with increased mortality. During the 7 year follow up period, 100 of the women died; women in the lowest quartile (<15.3 ng/ml 25(OH)D) (38.25 nmol/L) were at higher risk compared to women in the highest quartile (>27.0 ng/ml 25(OH)D)(67.5 mmol/L) (Hazards Ratio 2.45, 95%CI 1.12-5.36, $P$ = 0.02 in a multi-variate Cox proportional Hazards model adjusting for demographics, season, supplement use and conventional risk factors). Women in the lowest quartile were more likely to be black, not be taking vitamin D supplements and have a higher BMI and lower levels of physical activity. Addition of PTH, calcium and serum 1,25(OH)D2 levels to the model did not substantially change the relationship between serum 25(OH)D and mortality (HR 2.68, 95%CI 1.17-6.12, $P$ = 0.019).

75. A further study of data from NHANES was conducted by Ford *et al*., (2011). In this study, data from the NHANES mortality study from 2002-2004 was analysed, with mortality being compiled in 2006 (mean follow up 3.8 y). Of the 7531 participants aged ≥ 20 y, 347 died. The mean unadjusted concentrations of vitamin D were 54.1 nmol/L (21.7 ng/ml) among the participants that died, compared to 60.7 nmol/L (24.3 ng/ml) in the survivors ($p$= 0.002). After adjustment for socio-demographic factors, the hazard ratios were 1.65 (95%CI 1.13-2.40) with a concentration of < 50 nmol/L (20 ng/ml) 25(OH)D compared to those with a concentration ≥ 75 nmol/L (30 ng/ml). 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 25(OH)D was 0.93 (0.86-1.01). The HRs did not vary by gender or among the three major racial or ethnic groups. As the regression models were adjusted for more variables, the association diminished.

Table 9. Hazard Ratios of death referent to the highest quartile of serum 25(OH)D from Ford *et al*., (2011)

| | | Model 1* HR (95%CI) | Model 2[§] HR (95%CI) | Model 3[†] HR (95%CI) | Model 4[‡] HR (95%CI) | Model 5[¶] HR (95%CI) |
|---|---|---|---|---|---|---|
| 25 OHD nmol/L | Deaths | | | | | |
| 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 as model 1 + gender, race/ethnicity,

[†] Model 3 as model 2 + educational status, smoking status, alcohol intake, leisure time physical activity, vitamin and mineral supplement use,,

[‡] Model 4 as model 3 + blood pressure, a range of biochemical parameters (including serum Ca) and waist circumference,

[¶] Model 5 as model 4 +history of cardiovascular disease and/or diabetes

In ng/mL the quartiles are: 2.8-18, 18-24, 24-30, ≥30.

As with the 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 is a suggestion that the beneficial effect diminishes as serum levels increase, with the HR being higher in the top quartile than the third quartile. The authors concluded that the findings gave only limited support to an inverse relationship between serum vitamin D levels and all cause mortality; they did not consider the effect of higher levels.

76. Zitterman *et al*., (2012) conducted a meta-analysis to examine the relationship between vitamin D deficiency and mortality risk. For highest vs lowest categories of 25(OH)D, the summary RR or mortality was 0.71 (95%CI 0.50-0.91). The studies included those by Visser *et al*., 2006; Jia *et al*., 2007, Melamed *et al*., 2008 and Semba *et al*., 2009 as above. In a parametric model, the estimated summary RRs (95% CI) of 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 (5, 10 and 20 ng/ml) serum 25 (OH)D respectively, from a median reference category of approximately 27 nmol/L (10.8 ng/ml). There was no significant decrease in mortality when an increase of 87.5 nmol/L(35 ng/ml) above the reference category occurred. The model (Fig 4 of the paper attached at Annex B indicates a U shaped response but with the RRs

being below 1 but on an upward curve which, if it continued would suggest an increased risk at levels above 87.5 nmol/L. It was concluded that a non-linear decrease in mortality risk was apparent as circulating 25(OH)D increased, with an optimal serum concentration of 75-87.5 nmol/L (30-35 ng/ml).

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

**SACN Review of vitamin D. Adverse effects of high levels.**
**All cause mortality**

Extract from the COT minutes of June 2012.

**Item 4: SACN Review of vitamin D. Adverse effects of high levels - TOX/2012/23**

14. The Scientific Advisory Committee on Nutrition (SACN) was in the process of reviewing its recommendations on vitamin D, and the COT had been asked to advise on possible adverse effects of high levels of vitamin D intake. An introductory paper (TOX/2011/19) had been considered by the Committee in June 2011.

15. At that meeting it had been agreed that both human and animal data should be considered, and that it might be necessary to assess safety at different levels of vitamin D intake, rather than establish a single tolerable upper level (TUL). It was agreed also that it might be necessary to consider the relation of adverse effects to specified blood levels of vitamin D as well as to vitamin D intakes.

16. It had further been agreed that a 2011 US Institute of Medicine (IOM) report[4] should be used as a bibliographic source, since it was a very recent publication which included helpful systematic reviews. However additional papers published after the IOM review would also be considered, together with those identified by the Secretariat during the review process as being of particular interest.

17. Members were now provided with a new paper on the topic (TOX/2012/23 and its annexes). It was noted that this would need to be updated to take account of newly available data. Given the large amount of information that was available, it would be important to focus only on that which was relevant to the advice that SACN required. The SACN review would cover all aspects of vitamin D, including the low vitamin D levels which were prevalent in certain population groups.

18. Members were informed that new data on blood levels of vitamin D in the UK population of adults and older children would be available (from the National Diet and Nutrition Survey) by the end of July 2012, with corresponding information for infants in 2013. This would be considered by SACN later in the review process. It was agreed that as the distribution of blood levels would be important to COT's consideration of risks, the information on blood levels that was currently available should be provided in the first instance.

[4] Institute of Medicine, Committee to Review Dietary Reference Intakes for Vitamin and Vitamin D, Food and Nutrition Board (2011). Dietary Reference values for calcium and vitamin D and Fluoride. Available at http://www.nap.edu/catalog/13050.html

19. It was planned that a draft of the SACN review would be published for public consultation in 2013, and that new information could still be included after that time. Some information was available on the patterns of supplement use and how it had changed over time. Food composition tables might also provide information on vitamin D intakes, although it was noted that there were uncertainties about the methodology of the different vitamin D assays used. Exposure measures had not been standardised, though some validation work was underway.

20. It was agreed to use a single set of units, gravimetric for intakes and molar for serum levels. Members noted that whether calcitriol, the active form of vitamin D, was a hormone was controversial, and agreed that in the context of TULs it should be referred to as a nutrient as its role was to mobilise calcium.

21. Many published studies concerned the effects of supplementation with vitamin D and calcium in combination, making it difficult to discern the separate impact of vitamin D. Where vitamin D and calcium were given together it would be useful to know what doses of calcium were used, since the Committee's interest was in the effects of vitamin D at normal levels of calcium intake. Information on the effects of hypercalcaemia and excess calcium in the absence of vitamin D supplementation had been included in the paper as it should help in deciding what is relevant or important when looking at vitamin D induced hypercalcaemia. While it was useful background, this need not be included in the final report if it did not contribute to the conclusions.

22. In the published reports of vitamin D toxicity, it had always been associated with supplementation, fortification or medical treatment, and it appeared not to occur in the general population through normal dietary intake. However, it would be worth considering whether levels of intake could be of concern where infants were given both vitamin drops and also infant formula that was fortified with vitamin D.

23. Data had not been provided on the extent of vitamin D formation from sunlight. This was thought to be subject to greater homeostatic control and the uptake and metabolism via this route differed compared to dietary intake. Vitamin D produced from sunlight entered the circulation directly, whilst vitamin D from food or dietary supplements underwent significant presystemic metabolism in the liver. Cases of vitamin D toxicity attributed only to UV exposure had not been documented. Some studies had attempted to control for sunlight by taking into account the seasons in which blood samples were taken and/or physical activity (presumably as an indicator of time spent outdoors) in their models. SACN would be considering the impact of sunlight on vitamin D status and information would be provided to COT in due course.

24. Little information was available on potential adverse effects of vitamin D intake during pregnancy. There were no case reports relating to pregnancy, and pregnant women were likely to be excluded from clinical trials.

25. Some studies had suggested that vitamin D was less available when Body Mass Index (BMI) was higher. It was noted that the better quality studies corrected

for BMI. Members were advised that SACN would be considering the influence of BMI in their review of vitamin D.

26. It was noted that the non-monotonic exposure-response relationships which had been observed for some end-points such as all-cause mortality, had a reverse J-shape rather than a U-shape, with higher risk in the lowest category of serum vitamin D levels than in the highest category. The elevation of risk at higher serum levels might reflect confounding (some types of illness leading to increased serum levels and also predisposing to earlier death), and was not necessarily causal.

27. Members considered that the J-shaped curve described for pancreatic cancer was of interest, but that the suggested relationships for other cancer endpoints were not convincing and that it would not be necessary to refer the question of carcinogenicity to the COC at this time. It was noted that the Medicines and Healthcare products Regulatory Agency (MHRA) were looking at this topic, and in particular at possible interaction between vitamin D and IGFBP-3 in the cell signalling and proliferation processes. This had been observed *in vitro* and while it was possible that it was an artefact, it could also be a real effect. It was suggested that the World Cancer Research Fund (WCRF) continuous update could provide information on vitamin D and cancer.

28. Members agreed that it would be useful to consider the findings of Cochrane reviews on vitamin D, but that care should be taken to ensure that findings from primary research were not “double counted”.

29. It was agreed that hypercalcaemia could be a useful endpoint on which to base a TUL, but that it would be important to distinguish free ionised calcium, which drives toxicity, from total calcium which includes calcium bound to protein. Ionised calcium was under tight regulatory control. Albumin was not saturated and it was likely that bound calcium would predominate. Although, hypercalcuria could be considered as an outcome where it had been measured, it was harder to interpret (unless assessed in 24 hour samples) because of uncertainties about the extent of dilution from concomitant excretion of water. In addition, urinary calcium sometimes increased when serum calcium did not. The balance of blood and urinary calcium might be important.

30. Members doubted whether older people should be considered a potentially vulnerable group because of reduced kidney function. It was noted that kidney disease could reduce the metabolism of vitamin D to its active form, and this would tend to protect against toxicity. No other potentially vulnerable groups were identified. It was unclear if lifetime exposure to high levels of vitamin D increased vulnerability.

31. It was asked whether any genetic polymorphism might be relevant to toxicity. This issue had not been addressed in paper TOX 2012/23, but SACN would be looking at polymorphisms.

32. At present it was not possible to determine whether a TUL could be established either for total or for supplemental intake. Similarly it was not possible to

conclude whether vitamin D blood levels or intakes should be used in any advice. Further information would be needed on serum levels and their relationship with intake to draw any conclusions on this.

33. The COT's provisional position would be discussed by the SACN at their September meeting, either as a provisional position paper or as detailed minutes.

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

SACN Review of vitamin D. Adverse effects of high levels. All cause mortality

Schöttker B, Ball D, Gellert C, Brenner H. Serum 25-hydroxyvitamin D levels and overall mortality. A systematic review and meta-analysis of prospective cohort studies. Ageing Res Rev. 2013a, 12(2) 708-718.

For copyright reasons this paper is not attached to the publicly available version of TOX/2013/33