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TOX/2024/45

Committee on the Toxicity of Chemicals in Food, Consumer Products and the Environment

Discussion paper on the effects of Calcidiol supplementation during pregnancy

Introduction

1. In 2019, The Scientific Advisory Committee on Nutrition (SACN) agreed to conduct a risk assessment on nutrition and maternal health focusing on maternal outcomes during pregnancy, childbirth and up to 24 months after delivery; this would include the effects of chemical contaminants and excess nutrients in the diet.

2. SACN agreed that, where appropriate other expert Committees would be consulted and asked to complete relevant risk assessments e.g., in the area of food safety advice to support their review. Therefore, the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) was asked to consider whether exposure to excess vitamin D would pose a risk to maternal health, as part of this review. A Statement on the potential effects of excess vitamin D intake during preconception, pregnancy and lactation was published by the COT in 2022. Following a discussion of the matter arising agenda item at the COT meeting of December 2022, the COT agreed that calcidiol should also be considered as an Annex to the Statement on the potential effects of excess vitamin D intake during preconception, pregnancy and lactation. This was on the basis that calcidiol is a more potent form of vitamin D_2 and D_3 and its availability on the market is increasing.

3. The toxicity and biological function of vitamin D and its status in pregnancy has been discussed in the Statement on the potential effects of excess vitamin D intake during preconception, pregnancy and lactation and therefore will not be discussed in detail in this paper.

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Background

4. Calcidiol is a novel source of vitamin D_3 (cholecalciferol), which is formed via chemical synthesis from cholestatrienol (ACNFP, 2024). Calcidiol is also known as calcidiol monohydrate, 25-hydroxycholecalciferol monohydrate (25(OH)D_3 monohydrate) (EFSA, 2023), calcifediol or 25-hydroxyvitamin D (25(OH)D), with the latter two being the form used in supplementation (Biondi et al., 2017). Calcidiol is a synthetic form of 25(OH)D, which is an inactive precursor to the biologically active form of vitamin D known as 1,25-dihydroxyvitamin D (1,25 (OH)2D) and thus is commonly referred to as a pre-hormone (Vieth, 2020).

5. Calcidiol is more hydrophilic and has a shorter half-life than cholecalciferol (vitamin D_3) whilst causing a rapid and sustained rise in serum 25(OH)D levels (Navarro-Valverde et al., 2016). This is due to differences in vitamin D_3 absorption and hydroxylation in the liver, with calcidiol not requiring bile acids, which results in faster and more efficient absorption into systemic circulation (EFSA, 2022).

6. Calcidiol has been reported to be three to six times more potent than supplemental vitamin D_3, meaning that lower doses of calcidiol are required to achieve the same serum 25(OH)D levels as vitamin D_3 (Veith, 2020; Nishishinya, 2022). Other reports have showed 10 times more vitamin D_3 than calcidiol is needed to increase serum 25(OH)D levels to equivalent serum concentrations (Stamp et al., 1977).

7. As Vitamin D and calcidiol are not equipotent there is no universal agreement by regulatory authorities on the conversion factor of calcidiol to vitamin D_3 in international units (IU) (Gázquez et al., 2022). However, conversion factors of 1.4- to 5-fold have been estimated by Cashnman et al., 2012 and Rossini et al., 2005) The European Commission (EC) asked the European Food Safety Authority (EFSA) to derive a conversion factor for calcidiol to vitamin D_3 and the Panel on Nutrition, Novel Foods and Food

Allergens (NDA) Panel derived a conversion factor of 5 that was established by the Panel on Additives and Products or Substances to convert calcidiol to vitamin D in animal Feed (EFSA, 2023a). EFSA also considered vitamin D equivalents (VDE) which is the expression of the “biological value of substances with vitamin D activity”. EFSA determined 1 ug of VDE to be equivalent to 0.4 µg of calcidiol, which in turn is equivalent to 40 IU.

8. However, after an updated exposure assessment in response to a request from EirGen Pharma Ltd to revise their previous opinion, EFSA proposed a conversion factor of 2.5 for the intake of calcidiol to VDE in December 2023, based on a systematic review of 10 randomised clinical trials (RCT). In these RCTs the effects of weekly and daily doses of calcidiol at 20 and 25 µg/day were compared to Vitamin D_3 on serum 25(OH)D levels over a 6-week period. At doses of 20 µg/day the mean relative bioavailability of calcidiol was 2.02-fold higher than vitamin D_3, whereas at 25 µg/day the mean relative bioavailability of calcidiol was 1.31-fold higher than vitamin D_3. However, the mean relative bioavailability of calcidiol was 2.4-fold higher than vitamin D_3 in EFSA’s meta-analysis including all available RCTs. In two RCTs that used reported doses of vitamin D_3 at 60 µg/day the mean relative bioavailability of calcidiol was 2.11-fold higher than vitamin D_3. (EFSA, 2023b).

9. The UK Advisory Committee for Novel Foods and Processes (ACNFP) also agreed with EFSA’s conversion factor of 2.5 (ACNFP, 2024). Based on the ACNFP review of a calcidiol application submitted by DSM Nutritional Products Ltd the FSA and FSS concluded calcidiol as “safe under the proposed conditions of use and does not pose a safety risk to human health” (ACNFP, 2024).

Toxicokinetics

10. Calcidiol has the same identity as the primary metabolite of vitamin D_3 which is 25-hydroxycholecalciferol, also known as calcifediol (ACNFP, 2024). In humans both vitamin D_2 and vitamin D_3 are converted into this primary metabolite in the liver, and then converted to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) in the kidneys and other tissues (Perez-Lopez et al., 2015).

11. Calcidiol is more hydrophilic and has a shorter half-life than vitamin D_3 which results in more rapid and sustained increase in serum 25(OH)D (Navarro-Valverde et al., 2016). The half-life of calcidiol has been reported by Barger-Lux et al., (1998) to be 19 days in a study in healthy young men and was estimated to be 13.4 days in healthy males aged 18-23 years by Jones et al., (2012). Vicchio et al., (1993) estimated a half-life of calcidiol of 10 days using a liquid chromatography/thermospray mass spectrometry method (Brandi and Minisola et al., 2013).

12. In their safety assessment of calcidiol as a novel food for use in food supplements, the ACNFP had not considered whether the use of calcidiol (a metabolite of vitamin D_3) would affect the downstream metabolism and homeostatic regulation of circulating vitamin D_3 metabolite levels. However, based on data submitted by DSM Nutrition Ltd it was concluded that “the applicant did not give reason to believe that 25-hydroxycholecalciferol" (also known as calcidiol) “as a novel food would be metabolised differently from 25-hydroxycholecalciferol coming from other dietary sources or via this pathway, or that it would have wider impacts on feedback regulation, related pathways or vitamin D homeostasis.” Ultimately, the ACNFP stated that calcidiol has the same identity as the endogenous metabolites of vitamin D_3 (i.e. 25-hydroxycholecalciferol, calcifediol) and there is no evidence to suggest that they would behave differently in the body.

13. However, the ACNFP did note that information from literature suggested potential changes in metabolism in pregnant and lactating women

(ACNFP, 2024). As discussed in the Statement on the potential effects of excess vitamin D intake during preconception, pregnancy and lactation, “a number of studies have reported uniquely high levels of 1,25(OH)2D during pregnancy; the conversion of 25(OH)D to 1,25(OH)2D during the first trimester (12 weeks of pregnancy) results in a doubling of 1,25(OH)2D levels, and that levels continue to rise 2- to 3-fold until delivery”. Although, this increase in 1,25(OH)2D occurs without onset of hypercalciuria or hypercalcemia. Furthermore “the increase in 1,25(OH)2D observed during pregnancy is not continued throughout lactation and “Pregnant women with normal placental function but non-functional renal enzyme 1-α-hydroxylase fail to increase circulating $1,25(OH)_2D_3$ during pregnancy” (COT, 2022).

14. The ACNFP commented that data from literature supplied by the applicant reported serum $1,25(OH)_2D_3$ levels to be similar in pregnant and lactating women in response to vitamin D supplementation were similar in pregnant and lactating women and in non-pregnant or non-lactating women (Institute of medicine, 2011).

Toxicity

15. As discussed in the Statement on the potential effects of excess vitamin D intake during preconception, pregnancy and lactation the main adverse effects from excessive amounts of all forms of vitamin D including calcidiol (COT, 2022) are hypercalcaemia and hypercalciuria. These adverse effects have been described as infrequent and often a result of doses higher than recommended guidelines and for long durations (Robbins et al., 2022).

16. The following studies discussed below were derived from multiple sources including an ACNFP Committee Advice Document (CAD), an EFSA opinion on the “Safety of calcidiol monohydrate produced by chemical synthesis as a novel food pursuant to Regulation (EU) 2015/2283” (EFSA, 2021), and a literature search conducted by the FSA secretariat using the search terms listed on page 45.

Genotoxicity

17. The ACNFP reported the genotoxicity data submitted by the applicant (DSM Nutritional Products Ltd) in their assessment on the safety of calcidiol as a novel food for use in food supplements. A reverse mutation assay conducted in line with OECD No. 471 submitted by the applicant demonstrated calcidiol, at concentrations of 3, 10, 33, 100, 333, 1,000, 2,500, 5,000 µg/plate in experiment 1 and concentrations of 10, 33, 100, 333, 1,000, 2,500 and 5,000 in experiment 2, did not increase the number of revertant colonies and therefore is not mutagenic (Wöhrle and Sokolowski, 2013).

18. The ACNFP also received further data from the applicant on an *in vitro* mutagenicity test which was conducted in line with OECD No. 490. Experiment 1 tested calcidiol at concentrations of up to 7.5 and 25 µg/ml in the presence and absence of S9 mix. In experiment 2 calcidiol was tested at concentrations of up to 5 µg/ml in the absence of S9 mix. Calcidiol was found to not be mutagenic in both experiments (Remus and Verspeek-Rip, unpublished report, 2016).

19. The applicant also submitted results of an *in vivo* micronucleus test to the ACNFP. The test was in line with OECD No. 474. Results showed no increase in micro nucleated erythrocytes in treated animals, indicating that the test substance was not genotoxic in this test (Verbaan and Remus, 2016). Further results submitted by the applicant to the ACNFP were from an in vitro chromosome aberration test conducted in line OECD No. 473 (Weber and Schulz, 2005). In two experiments the highest concentration applied was 100 µg/mL of calcidiol. Neither experiment showed a statistically significant or biologically relevant increase in the number of cells carrying structural chromosomal aberration. Calcidiol was therefore concluded not to be clastogenic (Weber and Schulz, 2005).

20. The studies discussed above (Wöhrle and Sokolowski, 2013; Weber and Schulz, 2005; and Verbaan and Remus, 2016) were also discussed by EFSA in their opinion on the “Safety of calcidiol monohydrate produced by

chemical synthesis as a novel food pursuant to Regulation (EU)2015/2283". Based on these genotoxicity studies EFSA concluded that calcidiol as a novel food was of no concern regarding genotoxicity (EFSA, 2021).

Animal studies

21. Sub-chronic toxicity data obtained from primary research publications were also submitted to the ACNFP by DSM Nutritional Products Ltd in support of their application for calcidiol as a novel food for use in food supplements. In a 14-day study, male rats were orally administered vitamin D_3 at doses at 10-fold intervals from 0.65 - 6,500 nmol or calcidiol in Wesson oil at doses of 0.46 - 4,600 nmol/day. The average daily doses were estimated to be 2.3 - 22,750 µg/kg bw/day for vitamin D_3 and 1.7 - 16,770 µg/kg bw/day, for calcidiol. Study authors reported "signs of excessive intake of vitamin D_3" were observed at doses of vitamin D_3 at 2,275 and 22,750 µg/kg bw/day and calcidiol at 16,770 µg/kg bw/day (Shepard and DeLuva, 1980). Death occurred in 9/10 rats administered vitamin D_3 at 22,750 µg/kg bw/day. Other adverse effects included reduced plasma phosphorus concentrations, hypercalcaemia and greyish-white mottling of the kidneys (consistent with calcification) at doses of vitamin D_3 at 2,275 µg/kg bw/day and calcidiol at 16,770 µg/kg bw/day.

22. In a 90-day study conducted in line with OECD No. 408, male and female rats received oral doses of calcidiol equivalent to 0, 7, 20, 60 and 180 µg/kg bw/day in a powdered formulation. The formulation also contained antioxidant excipients of which many have biologically active potential which was not excluded given the absence of an excipient control. Mineralisation of the renal pelvis was observed in both sexes at doses of calcidiol of 20 µg/kg bw/day and above, and more specifically 7 µg/kg bw/day in females. However, study authors did not consider this effect as adverse due to the absence of clinical chemistry findings indicating kidney dysfunction. Study authors therefore proposed an equivalent NOAEL of calcidiol of 180 µg/kg bw/day (Thiel et al., 2007).

23. Further analysis of the histopathological findings from this study by Hard, 2014 concluded that mineralisation is more common in rats than any other species of laboratory animals. Also, the pattern of mineralisation observed is inconsistent with that of hypercalcaemia due to excess vitamin D_3. Hard, 2014 attributed this inconsistency to the hygroscopic nature of the test substance and possibly the nature of excipients used in the study whose effects could not be excluded because of the lack of an excipient control in this study.

24. Based on the reported findings by Thiel et al., 2007 the ACNFP stated that “the mineralisation observed indicates disruption of kidney function at all doses in female rats and in all but the lowest dose in males. It is not, therefore, possible to derive a NOAEL from this study. If the lowest dose used, 7 µg/kg bw/day, which caused renal mineralisation in female rats only, is taken as a Lowest Observed Adverse Effect Level (LOAEL) this yields a margin of safety of 49 for a 70 kg adult ingesting 25-hydroxycholecalciferol at a dose of 10 mg/day or 21 for a 15 kg child taking 5 mg/day”. Ultimately, the ACNFP concluded that “given the human safety data provided, this provides sufficient reassurance for the use of 25-hydroxycholecalciferol at the proposed doses in humans” (ACNFP, 2024).

Human studies and case reports

25. EFSA reviewed the safety of calcidiol monohydrate as a novel food intended for use in food supplements in 2021 and they reported that adults supplementing with 10 µg/day (as a novel food) their serum 25(OH)D levels remained below 200 nmol/L, which is considered to be in the normal range of 25 - 200 nmol/L (COT, 2014). It should be noted that “circulating levels of 25(OH)D in the blood are normally in the range of 25-200 nmol/L (COT, 2014) but Hollis, 2005 reported circulating levels of 135 to 225 nmol/L in sunny environments where clothing or cultural practices do not prevent sun exposure (COT, 2014)”. (COT, 2022)

26. The ACNFP safety assessment on an application for calcidiol as a novel food for use in food supplements reported that “several trials have compared the effectiveness of Vitamin D_3 and 25- hydroxycholecalciferol” (ACNFP, 2024). The ACNFP further reported that “No intoxication as measured by hypercalcemia has been reported in humans at serum 25-hydroxycholecalciferol levels below 500 nmol/L (Heaney, 2008, and Hathcock et al., 2007).”

27. In a study with 116 healthy men that received calcidiol at doses of 10, 20 or 50 mg/day for a duration of 4 weeks, calcidiol was reported to be safe up to 50 mg/day. Treatment with calcidiol increased circulating serum 25(OH)D levels by 40, 76 and 206 nmol/L in those receiving the respective doses of 10, 20 and 50 mg/day. However, treatment with calcidiol did not increase 1,25(OH)D serum levels (Barger-Lux et al., 1998).

28. The ACNFP also reported findings from an unpublished randomised, controlled, double blind pharmacokinetic study. The response of serum 25(OH)D levels to calcidiol were compared to vitamin D_3 supplementation. The ACNFP reported findings from the Kunz et al., (2016) study that 20 µg/day could be safely administered for up to 6 months (Kunz et al., 2016). In addition to this being an unpublished study, no further information on the study was reported in the ACNFP opinion. However, EFSA, 2021 also cited this study, and reported findings of no changes in fasting 2-h morning urine calcium/creatine ratio over the 6-month study duration. Further findings from the study, as reported by EFSA, 2021, were that the 15 and 20 µg/day calcidiol dose groups had higher mean 24-hour urine calcium levels compared to the 20 µg/day vitamin D dose group. However, the urine calcium levels in both groups remained <300 mg/24 h which was labelled as “a range of no concern” by study authors (EFSA, 2021 citing Kunz et al., 2016).

29. In a randomised, double-blind study with 59 men and women aged ≥65 years, study authors concluded calcidiol to safely elevate serum 25(OH)D_3 level. Participants received either 5, 10 or 15 µg of calcidiol or 20 µg/vitamin D_3 and no cases of hypercalcemia occurred throughout the study duration.

Adverse events were reported, however, none of them resulted in termination of the study and were deemed as unrelated to the treatment administered (Vaes et al., 2018).

30. In another study investigating the metabolic changes after calcidiol administration, no safety concerns were reported. Participants were 18 healthy women aged 24-72 years and received calcidiol at doses of 500 µg/month over a 4-month period. However, it should be noted that this was not a safety study intentionally assessing adverse effects (Russo et al., 2011).

31. Many of the studies investigating the safety of calcidiol have been conducted in menopausal women. They have also been conducted in vitamin D deficient individuals (defined as serum 25(OH)D levels being <30 ng/mL by various study authors) due to calcidiol's higher potency than vitamin D and potential treatment for vitamin D deficiency (Quesada-Gomez, et al., 2023).

32. The safety and efficacy of calcidiol was studied in 45 postmenopausal women with vitamin D deficiency. Participants received doses of calcidiol at 0.266 mg/month (266 µg/month) over a 2-year duration. Study authors reported that mean 25(OH)D levels at month 24 of calcidiol administration was not significantly different to mean 25(OH)D levels at month 12. No significant changes in serum ionised calcium or creatinine were observed, and there were no significant changes in bone alkaline phosphatase serum levels between 12 and 24 months. Study authors concluded that there were no safety concerns with long term administration of calcidiol as only one minor adverse event of mild dyspepsia (i.e. indigestion) was reported (Occhiuto et. al., 2024).

33. In a double-blind randomised controlled trial, the safety and efficacy of calcidiol was compared with vitamin D_3 in 303 participants. Participants were vitamin D deficient postmenopausal women and received calcidiol in the form of capsules at a dose of 266 µg/month for 4 months. After this period participants were administered a placebo for 8 months followed by vitamin D_2 at 625 µg/month for 12 months. The highest serum 25(OH)D level reported

was 60 ng/mL. No deaths were reported, only one participant had an adverse event that led to her withdrawal from the study and 8 participants reported at least one serious adverse event. However, study authors did not elucidate what these adverse events were and whether they were related to administration from calcidiol or vitamin D. Study authors concluded calcidiol to be safe in postmenopausal women as no serious adverse events were attributable to either calcidiol or vitamin D_3 (Pérez-Castrillón et al., 2020).

34. Calcidiol was demonstrated to be safe at daily doses of 20 µg/day, 40 µg/day or 125 µg/week for 3-months in 87 post-menopausal vitamin D deficient women aged 55 years and over. The levels of 25(OH)D remained within the study authors safety window of 30 to 100 ng/mL after 14 days of treatment and no significant changes in markers for calcium and phosphate metabolism or bone turnover were reported. Furthermore, serum 25(OH)D levels were similar in both groups receiving 20 µg/day or 125 µg/week, serum levels were 49.3 and 46.4 ng/mL, respectively. However, 25(OH)D levels doubled in the group receiving 40 µg/day, with serum levels reaching 74.8 ng/mL. Ultimately, there was no difference in serum 25(OH)D levels between 30 and 90 days of treatment and study authors concluded that this indicated a plateau phase in short to medium term calcidiol administration (Minisola et al., 2017).

35. An earlier study administering calcidiol at 140 µg/week in a single dose resulted in serum 25(OH)D near 50 ng/mL (Jetter et al., 2014), which have been linked to non-classical adverse events and side effects of vitamin D supplementation such as falls or increased in bone turnover markers (Jetter et al., 2014, Rossini et al., 2012a, Rossini et al., 2012b).

36. In 20 healthy postmenopausal women aged 50-70 years receiving oral administrations of calcidiol at 20 µg/day over a 4-month period, no adverse effects were reported. As part of the study's compliance and safety regimen, adverse events were monitored, and serum calcium was measured. No adverse events were reported, and serum calcium levels were stable and did not exceed the upper end of the reference range (i.e. 2.19 - 2.60 nmol/L)

throughout the 4 month follow up period. Study authors also reported “a safe, immediate sustained increase in 25(OH)D serum levels. However, this study was not a safety study set out to assess safety indications, but a study comparing the effectiveness of calcidiol and vitamin D_3 on serum 25(OH)D levels, lower extremity function and, innate immunity (Bischoff-Ferrari et al., 2012).

37. Graeff-Armas et al., 2020 also reported no safety concerns of calcidiol in a randomised, double-blind study that administered doses of 10, 15 and 20 µg/day for 6 months. The 91 participants of this study were healthy men and postmenopausal women aged 50 years and over.

38. It should be noted that this study was not a safety study but an efficacy study comparing the pharmacokinetics of calcidiol to vitamin D_3. However, safety and tolerability assessments were conducted that assessed serum calcium, creatine, 1,25(OH)2D and parathyroid hormone. Complete metabolic panels, complete blood counts and adverse effects were reported. Study authors reported no significant changes to serum calcium, creatine, 1,25 $(OH)_2D$ from calcidiol administration. There was a statistically significant increase from baseline in 24-hour urinary calcium excretion at 20 µg/day in the calcidiol dose group compared to the vitamin D_3 group. Overall, safety laboratory parameters remained within normal reference ranges. None of the adverse events reported were considered related to calcidiol or vitamin D_3 and did not result in participant withdrawal. Therefore, study authors concluded that “the intervention was well tolerated and safe” over the 6-month dosing period (Graeff-Armas et al., 2020).

39. Further studies reporting no safety concerns of calcidiol include a convenience study conducted by Navarro Valverde et al., (2016). Forty postmenopausal women (average age = 67 years) with osteoporosis received calcidiol orally for a period of 12 months at doses of 20 µg/day, 266 µg/week or 266 µg every other week. The participants of this study had pre-existing vitamin D deficiency with 25(OH)D levels at or below 37.5 ± 5 nmol/L. It should be noted that, the aim of this study was to compare the potency of

vitamin D_3 and calcidiol, and not to assess safety. Also, the study authors did not describe any safety and compliance regimen undertaken, including the noting of adverse events and reported side effects.

40. In a phase I clinical trial assessing the safety and efficacy of calcidiol, it was concluded to be safe and effective in the young adult population. There were 101 participants aged 18-50 years with a mean age of 29.8 years. Participants received calcidiol over a 4-month period either monthly or every 2 weeks depending on their vitamin D deficiency. Participants with mild to moderate deficiency (defined as serum 25 (OH)D of 10 - <20 ng/mL by study authors) received calcidiol monthly. However, those with severe deficiency (defined as serum 25(OH)D of <10 ng/mL by study authors) received calcidiol every 2 weeks (Guerra López et al., 2024).

41. Safety results from the clinical trial showed only one participant developed 25(OH)D plasma levels >60 ng/mL whose levels retuned to 10 ng/mL after termination of calcidiol treatment. Nine of the adverse events reported were considered to be potentially related to the study and were reported by 5% of participants receiving calcidiol monthly. These adverse events were of mild or moderate severity and included headache, non-clinically relevant increases in parathyroid hormone, nausea, abdominal discomfort, decreased appetite and diarrhoea (Guerra López et al., 2024).

42. No safety concerns of calcidiol administration were reported in a 16-week randomised controlled trial. There were 35 participants aged 18 years and over with vitamin D deficiency (defined as serum 25(OH)D of ≤20 ng/mL by study authors). Participants received either calcidiol at 60 µg/day or vitamin D_3 at 20 µg/day. Serum calcium and urinary calcium excretion were used as parameters of safety, and no significant changes from baseline levels were reported in these parameters. Furthermore, there were no reports of hypercalcemia, hypercalciuria or nephrolithiasis (kidney stones). Participants were also asked about adverse events, however, the occurrence and severity

of any adverse events were unreported by the study authors (Shieh et al., 2017).

43. In a randomised study evaluating the efficacy and safety of calcidiol in 50 women with osteoporosis and osteopenia, calcidiol was reported to have long-term safety, by the study authors, at doses of 20 µg/day and 30 µg/day. Participants were aged 55-70 years and had 25(OH)D levels of 10-20 ng/mL and therefore vitamin D insufficient (defined by the study authors and the Institute of Medicine as 12-20 ng/ml) (Ross et al, 2011). Adverse events were monitored at baseline, 15, 30, 60, 90 and 180 days after the start of treatment. No adverse events were reported by the study authors. None of the participants presented hypercalcemia, and only one participant receiving calcidiol at 30 µg/day presented borderline hypercalciuria (i.e. urinary calcium of 320 mg/24/hour). However, study authors stated that one of the key findings was “the confirmation about long-term safety of calcifediol and the lack of toxic effects” (Gonnelli et al., 2021).

44. No adverse events from calcidiol were reported in a randomised, placebo-controlled, double-blind study comparing the effectiveness of calcidiol to vitamin D_3 in 56 participants. Participants were healthy white men and women and aged 50 years and over, and either consumed placebo, 20 µg/day of vitamin D_3 or 7 or 20 µg/day of calcidiol over a 10-week period. There were no cases of hypercalcemia in the study, serum albumin-corrected calcium concentrations did not exceed 2.6 nmol/L (Cashman et al., 2012).

45. Other studies assessing the efficacy and safety of calcidiol include a longitudinal cohort study with 123 HIV-infected patients, 24% of which were female. Patients received monthly calcidiol doses of 16,000 IU/month (400 µg/month) and showed no signs of clinical toxicity. Serum 25(OH)D levels did not exceed >100 ng/ml, although patients had pre-existing vitamin D deficiency (defined as 25(OH)D of <10 – 29.9 ng/mL by study authors). Furthermore, there were no reported cases of hypercalcemia (defined as serum calcium of >10.5 ng/mL by study authors), gastrointestinal intolerance

or headaches. Therefore, study authors concluded the monthly dose of calcidiol was safe in vitamin D deficient HIV-infected patients (Banon et al., 2015).

Preconception

46. There are currently no data available on the toxicological effect of calcidiol supplements during preconception. The Statement on the potential effects of excess vitamin D intake during preconception, pregnancy and lactation had also reported "no information on the effect of excess vitamin D during preconception" (COT, 2022).

Pregnancy

47. The data available on the toxicological effects of calcidiol supplements during pregnancy are limited. However, in a phase I clinical trial assessing the safety and efficacy of calcidiol in 101 participants aged 18-50 years with a mean age of 29.8 years. Participants received calcidiol over a 4-month period either monthly or every 2 weeks depending on their vitamin D deficiency. Participants with mild to moderate deficiency (defined as serum 25(OH)D of 10 - <20 ng/mL by study authors) received calcidiol monthly. However, those with severe deficiency (defined as serum 25(OH)D of <10 ng/mL) received calcidiol every 2 weeks. At the end of the treatment period there was one reported case of pregnancy in the placebo group. However, due to the participant's vitamin D deficiency (serum 25(OH)D of <15 ng/mL) she was prescribed calcidiol by her obstetrician outside of the study, a dose of 0.255 mg/month for a duration of 54 days. Study authors confirmed the participant to have a normal delivery with no maternal or birth-related complications. Overall, study authors concluded calcidiol to be safe in doses trialled in this study (Guerra López et al., 2024).

48. Further information on the adverse effects of vitamin D during pregnancy have been addressed in the Statement on the potential effects of

excess vitamin D intake during preconception, pregnancy and lactation (COT, 2022). These adverse effects include hypercalcaemia and hypercalciuria in pregnant women which may result in foetal morbidity. Neonatal hypercalcemia and or neonatal morbidity may also occur". Other possible adverse effects include high maternal blood pressure (COT, 2022).

Lactation

49. There are currently no data available on the toxicological effects of calcidiol during lactation. However, the limited evidence for the adverse effects of high vitamin D_2 and D_3 consumption during lactation have been addressed in the Statement on the potential effects of excess vitamin D intake during preconception, pregnancy and lactation, where "possible hypercalciuria" was reported as the only adverse effect (COT, 2022).

Health based guidance values

50. For VDE, EFSA established a tolerable upper intake level (UL) of 100 µg VDE/day, for adults (including pregnant and lactating women) and adolescents aged 11-17 years (EFSA, 2023a). The UL covers all sources of dietary intake.

51. For vitamin D the same tolerable upper limit (TUL) of 100 µg was established for adults (including pregnant and lactating women (EFSA 2012), which the COT agreed with (COT, 2022).

52. EFSA considered calcidiol supplements safe up to intake levels of 10 µg/day for adolescents (≥11 years old) and adults (including pregnant and lactating women) which corresponds to 25 µg VDE/day with their applied 2.5-fold conversion factor.

53. However, applying the 2.5 conversion factor to the TUL of 100 µg/day for vitamin D, would give calcidiol an adjusted upper intake of 40 µg/day for

adults (ACNFP, 2024). This is greater than EFSA'S proposed safe intake level 10 µg/day,

Exposure assessment

Occurrence of calcidiol in food

54. Calcidiol, may be present in some foods of animal origin such as milk, butter, eggs, fish, meat and offal in the form of 25-hydroxycholecalciferiol (25(OH)D3) or 25-hydroxyergocalciferol (25(OH)D2). Calcidiol in the form 25-hydroxyergocalciferol has been reported in whole milk, butter and in some meat and offal (Ovesen et al., 2003; Jakobsen and Saxholt, 2009).

55. Occurrence of calcidiol in 11 food sources was reported in EFSA's 2021 paper 'Safety of *calcidiol monohydrate* produced by chemicals synthesis as a novel food pursuant to Regulation (EU)2015/2283'. These levels have been summarised and can be found in Table 1.

Table 1. Foods containing calcidiol (Adapted from page 11 of EFSA, 2021).

Food	Form of calcidiol	Concentration (µg /kg)
Semi-skimmed milk	25-hydroxycholecalciferol	0.042
Whole milk	25-hydroxyergocalciferol	0.031
Butter	25-hydroxycholecalciferol,	0.96
Butter	25-hydroxyergocalciferol	0.58
Egg yolks	25-hydroxycholecalciferol	5 – 12
Salmon flesh	25-hydroxycholecalciferol	1.1
Raw trout	25-hydroxycholecalciferol	2.2
Pork cuts	25-hydroxycholecalciferol	0.7 – 1.4
Pork rind	25-hydroxycholecalciferol	3.4
Pork liver	25-hydroxycholecalciferol	4.8

Cow Kidney	25-hydroxycholecalciferol	5.1 – 9.8
Beef Liver	25-hydroxyergocalciferol	1.7

Food consumption

56. The following exposure assessments for calcidiol in food are based on consumption data from the National Diet and Nutrition Survey (NDNS) (Bates et al., 2014, 2016, 2020; Roberts et al., 2018); however, it is important to note that the NDNS does not provide data for pregnant or lactating women. Therefore, data presented below are based on women of childbearing age (16-49 years) and consumption data may not be entirely representative of the maternal diet, specifically in liver food groups due to National Health Service (NHS) recommendations that pregnant women should not consume liver or liver products (NHS, 2024). Evidence suggests that some foods and nutrients may be under-reported to a greater extent than others, and some may be overreported, but there is no information available on the level to which different foods are misreported in the NDNS in this group.

57. Consumption data were generated for all 11 food groups in Table 1 including both whole foods and recipes; these data can be found in Annex A. Table A1 provides acute consumption data and Table A2 provides chronic consumption data. Both tables summarise the mean and 97.5th percentile consumption per food group, for women of childbearing age.

Milk

58. A search within the recipes database of the NDNS (Bates et al., 2014, 2016, 2020; Roberts et al., 2018) was conducted to retrieve both semi-skimmed milk, whole milk, and recipes containing milk which had been recorded in the survey. Other types of milk were excluded as this search was conducted based on the food groups described in Table 1.

Butter

59. Calcidiol has been detected in butter as both 25-hydroxycholecalciferol and 25-hydroxyergocalciferol (EFSA, 2021). Consumption data were retrieved for butter and recipes containing butter.

Egg Yolk

60. Both whole egg and yolk only consumption was included from the NDNS database to ensure that all egg yolk consumers were included. Foods containing egg white only were excluded from the assessment. The egg yolk makes up approximately 29.3% of the edible portion of a medium egg, and 28.7% of a large egg. The NDNS database does not specify the use of large or medium eggs therefore the figure was rounded to 29% (DH, 2012) and applied to whole eggs foods to give estimates for consumption specifically of egg yolks.

Salmon

61. Foods containing salmon in the NDNS database do not specify with or without skin, however the assumption has been made that recipes represent salmon flesh.

Trout

62. Due to a low number of consumers of trout in the NDNS database, an ‘all fish’ food group was used as proxy based on the assumption that trout is eaten in similar quantities to other types of fish such as cod and haddock.

63. It is important to note that whilst levels of Calcidiol were detected in raw trout, both canned and cooked fish and fish recipes were used within this exposure assessment as raw trout data were not available within the NDNS.

Pork

64. Calcidiol is present in in pork cuts, pork rind, and pork liver as 25-hydroxycholecalciferol. The NDNS database was used to retrieve recipes

containing varying forms of pork meat including pork belly, pork loin, sausages and bacon. Within the database, pork crackling was used to represent consumption of pork rind.

Beef kidney

65. Due to a low number of consumers of beef kidney in the NDNS database, an ‘all kidney’ food group was used as proxy based on the assumption that kidney from animals such as lamb and pork would be consumed similarly.

Beef Liver

66. For women of childbearing age, within the NDNS database there are no consumers of beef liver, therefore an ‘all liver’ food group was used as proxy based on the assumption that liver from animals such as chicken and lamb would be consumed similarly.

Exposure estimates from food

67. An exposure assessment was conducted using food groups and occurrence levels presented in Table 1 only. A summary of exposure estimates for each food at its corresponding occurrence level of calcidiol can be found in Table 2 and 3. Table 2 provides acute exposure estimates to calcidiol from food, and Table 3 provides chronic exposure estimates, for women of childbearing age. In these tables, acute and chronic exposures are presented for both mean and 97.5th percentile groups on a per person and per kilogram bodyweight basis.

Table 2: Estimated acute exposure to Calcidiol from food for women of childbearing age (16-49 years).

Food Groups	Type of Calcidiol	Level(s) detected (µg /kg)	Number of consumers	(ug/person/day) * Mean	P97.5	(ug/kg bw/day) * Mean	P97.5
Semi-skimmed milk	25-hydroxycholecalciferol	0.042	2083	0.0085	0.026	0.00013	0.00041
Whole milk	25-hydroxyergocalciferol	0.031	1333	0.0041	0.017	0.000063	0.00026
Butter	25-hydroxycholecalciferol	0.96	1736	0.015	0.049	0.00023	0.00074
Butter	25-hydroxyergocalciferol	0.58	1736	0.0092	0.029	0.00014	0.00045
Egg yolk	25-hydroxycholecalciferol	5.0 - 12.0	2128	0.17- 0.41	0.46 - 1.1	0.0025 - 0.0061	0.0072 - 0.017
Salmon	25-hydroxycholecalciferol	1.1	375	0.087	0.22	0.0013	0.0036
Trout	25-hydroxycholecalciferol	2.2	168	0.17	0.52	0.0026	0.0082

Pork cuts	25-hydroxycholecalciferol	0.7 - 1.4	1406	0.049 - 0.099	0.15 - 0.3	0.00072 - 0.0014	0.0021- 0.0044
Pork rind	25-hydroxycholecalciferol	3.4	69	0.053	0.21	0.00079	0.003
Pork liver	25-hydroxycholecalciferol	4.8	68	0.096	0.26	0.0013	0.0034
Cow Kidney	25-hydroxycholecalciferol	5.1 - 9.8	17**	0.077 - 0.15	0.14 - 0.27	0.0011 - 0.0021	0.0022 - 0.0042
Beef Liver	25-hydroxyergocalciferol	1.7	96	0.063	0.21	0.00093	0.0036

*Rounded to 2 s.f.

** Consumption or exposure estimates made with a small number of consumers may not be accurate. Where the number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers.

Table 3: Estimated chronic exposure to Calcidiol from food for women of childbearing age (16-49 years).

Food Groups	Type of Calcidiol	Level(s) detected (µg /kg)	Number of consumers	(ug/person/day) * Mean	P97.5	(ug/kg bw/day) * Mean	P97.5
Semi-skimmed milk	25-hydroxycholecalciferol	0.042	2083	0.0048	0.017	0.000071	0.00024
Whole milk	25-hydroxyergocalciferol	0.031	1333	0.002	0.01	0.000031	0.00016
Butter	25-hydroxycholecalciferol	0.96	1736	0.0066	0.024	0.0001	0.00038
Butter	25-hydroxyergocalciferol	0.58	1736	0.004	0.014	0.00006	0.00023
Egg yolks	25-hydroxycholecalciferol	5.0 - 12.0	2128	0.066 - 0.16	0.2 - 0.47	0.00098 - 0.0024	0.0032-0.0076
Salmon	25-hydroxycholecalciferol	1.1	375	0.025	0.059	0.00037	0.00098
Trout	25-hydroxycholecalciferol	2.2	168	0.047	0.16	0.0007	0.0022

Pork cuts	25-hydroxycholecalciferol	0.7 - 1.4	1406	0.016 - 0.033	0.057 - 0.11	0.00024 - 0.00048	0.00089 - 0.0018
Pork rind	25-hydroxycholecalciferol	3.4	69	0.015	0.053	0.00022	0.00079
Pork liver	25-hydroxycholecalciferol	4.8	68	0.028	0.09	0.0004	0.0013
Cow Kidney	25-hydroxycholecalciferol	5.1 - 9.8	17**	0.02 - 0.038	0.038 - 0.073	0.00027 - 0.00052	0.00055 - 0.001
Beef Liver	25-hydroxyergocalciferol	1.7	96	0.017	0.06	0.00026	0.00092

*Rounded to 2 s.f.

** Consumption or exposure estimates made with a small number of consumers may not be accurate. Where the number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers.

Milk

68. Acute exposure estimates derived for 25-hydroxycholecalciferol in semi-skimmed milk at a concentration of 0.042 µg/kg are 0.0085 µg/day and 0.026 µg/day mean and 97.5th percentile values, respectively. Chronic exposure estimates are 0.0048 µg/day and 0.017 µg/day mean and 97.5th percentile values, respectively.

69. Acute exposure estimates derived for 25-hydroxyergocalciferol in whole milk at a concentration of 0.031 µg/kg are 0.0041 µg/day and 0.017 µg/day mean and 97.5th percentile values, respectively. Chronic exposure estimates are 0.002 µg/day and 0.01 µg/day mean and 97.5th percentile values respectively.

Butter

70. Calcidiol in butter was detected as 25-hydroxyergocalciferol at a concentration of 0.58 µg/kg and as 25-hydroxycholecalciferol at a concentration of 0.96 µg/kg and.

71. At a concentration of 0.58 µg/kg acute exposures were 0.0092 µg/day and 0.029 µg/day mean and 97.5th percentile values, respectively. At a concentration of 0.96 µg/kg, acute exposure estimates are 0.015 µg/day and 0.049 µg/day mean and 97.5th percentile values, respectively.

72. Chronic exposure estimates at a concentration of 0.58 µg/kg are 0.004 µg/day and 0.014 µg/day mean and 97.5th percentile values, respectively. At a concentration of 0.96 µg/kg, exposure estimates are 0.0066 µg/day and 0.024 µg/day mean and 97.5th percentile values, respectively.

Egg yolk

73. In egg yolk, 25-hydroxycholecalciferol was detected at a range of 5.0 to 12 µg/kg. Acute exposure estimates range from 0.17 to 0.41 µg/day and 0.46 to 1.1 µg/day mean and 97.5th percentile values, respectively. Chronic

exposure estimates range from 0.066 to 0.16 µg/day and 0.2 to 0.47 µg/day mean and 97.5th percentile values, respectively. The highest exposure to calcidiol from food was noted from egg yolks.

Salmon

74. Acute exposure estimates to 25-hydroxycholecalciferol in salmon at a level of 1.1 µg/kg are 0.087 µg/day and 0.22 µg/day mean and 97.5th percentile values, respectively. Chronic exposure estimates are 0.025 µg/day and 0.059 µg/day mean and 97.5th percentile values, respectively.

Pork

75. 25-hydroxycholecalciferol was detected at a range of 0.7 to 1.4 µg/kg in pork cuts, 3.4 µg/kg in rind (crackling), and 4.8 µg/kg in pork liver.

76. Acute mean exposures range from 0.049 to 0.099 µg/day in pork cuts, 0.053 µg/day in rind (crackling), and 0.096 µg/day in pork liver. Acute exposure estimates at the 97.5th percentile range from 0.15 to 0.3 µg/day in pork cuts, 0.21 µg/day in rind (crackling), and 0.26 µg/day in pork liver.

77. Chronic mean exposures range from 0.016 to 0.033 µg/day in pork cuts, 0.015 µg/day in rind (crackling), and 0.028 µg/day in pork liver. Chronic exposure estimates at the 97.5th percentile range from 0.057 to 0.11 µg/day in pork cuts, 0.053 µg/day in rind (crackling), and 0.09 µg/day in pork liver.

Beef

78. 25-hydroxycholecalciferol was detected in beef kidney at a range of 5.1 to 9.8 µg/kg, whilst 25-hydroxyergocalciferol was detected at a level of 1.7 µg/kg in beef liver.

79. Acute mean exposures range from 0.077 to 0.15 µg/day in beef kidney, and 0.063 µg/day in beef liver. Acute exposure estimates at the 97.5th

percentile range from 0.14 to 0.27 µg/day in beef kidney, and 0.21 µg/day in beef liver.

80. Chronic mean exposures range from 0.02 to 0.038 µg/day in beef kidney, and 0.017 µg/day in beef liver. Chronic exposure estimates at the 97.5th percentile range from 0.038 to 0.073 µg/day in beef kidney, and 0.06 µg/day in beef liver.

Total exposure estimates from food sources

81. Estimated total exposures to calcidiol from 11 food sources (Table 1), in women aged 16-49 years, are presented in Tables 3 and 4 below. Due to a range of occurrence values for some food groups, these data have been presented as minimum and maximum exposure estimates. Exposure data from food sources containing calcidiol will be compared to the ACNFP TUL of 40 µg/day and EFSA's safe level of intake of 10 µg/day.

Table 3. Estimated total acute exposure to calcidiol from food sources (excluding supplements) in women aged 16-49 years.

Total calcidiol exposure (food sources)**	**(µg/person/day) * Mean**	**P97.5**	**µg/kg bw/day* Mean**	**P97.5**
Minimum	0.19	0.5	0.0028	0.008
Maximum	0.4	1.1	0.006	0.017

*Rounded to 2 s.f.

** Determined from a distribution of consumption of any combination of categories, rather than by summation of the respective individual mean / 97.5th percentile consumption value for each of the 11 food categories.

82. Women of childbearing age are estimated to have minimum acute calcidiol exposures of 0.19 and 0.5 µg/day for mean and 97.5th percentile

consumption, respectively. Maximum acute exposures are 0.4 and 1.1 µg/day for mean and 97.5th percentile consumption, respectively.

Table 4. Estimated total chronic exposure to calcidiol from food sources (excluding supplements) in women aged 16-49 years.

Total calcidiol exposure (food sources)**	**(µg/person/day) * Mean**	**P97.5**	**µg/kg bw/day* Mean**	**P97.5**
Minimum	0.082	0.24	0.0012	0.0038
Maximum	0.17	0.52	0.0025	0.0081

* Rounded to 2 s.f.

**Determined from a distribution of consumption of any combination of categories, rather than by summation of the respective individual mean / 97.5th percentile consumption value for each of the 11 food categories.

83. Women of childbearing age are estimated to have minimum chronic exposures of calcidiol at 0.082 and 0.24 µg/day mean and 97.5th percentile values, respectively. Maximum expsoures are 0.17 and 0.52 µg/day mean and 97.5th percentile values, respectively.

Exposure estimates from supplements

84. Calcidiol is currently available in supplemental form and may be used in future food fortification (Guo et al., 2017). Calcidiol is present in supplements in the form of calcifediol or 25(OH)D (Biondi et al., 2017).

85. Supplements aimed at adults were identified using online sources which supplied calcidiol in doses ranging from 10 to 20 µg/day. No supplements containing calcidiol were identified that were specifically aimed at pregnant and breast-feeding women.

86. Estimate calcidiol exposures from calcidiol-containing supplements are presented in Table 5. These exposure estimates assume that a 70.3 kg female between the ages of 16 to 49 consumes the supplement at the recommended dose for adults. The bodyweight of 70.3 kg was determined as the mean bodyweight of all females of childbearing age (16 to 49 years) within years 1-11 of the NDNS database.

87. The limited number of calcidiol-containing supplements available on the market are presented in Table 5, some of which are not available in the UK, but are able to be ordered online from international stores.

Table 5. Calcidiol exposure estimates for women of childbearing age consuming calcidiol-containing supplements*.

Supplement	**Calcidiol concentration per serving (µg)**	**Serving size (tablets/day)**	**Calcidiol exposure (µg/kg bw/day)****
VitamoreD - Vitamin D3 as Calcifediol	10	1	0.14
D.velop Tablets Adult	20	2	0.28
D.velop Gummies Adult	10	2	0.14
Bioclinic Naturals Opti Active D	10	1	0.14
Vitamin D DPrev Active	10	1	0.14

* based on a bodyweight of 70.3kg.

** Rounded to 2 s.f.

88. The supplements listed in Table 5 are generally aimed at adults although it should be noted that pregnant women may consume these supplements as many individuals are unaware of their pregnancy at the time

and may consume calcidiol-containing supplements that are of higher potency than vitamin D_2 and D_3 supplements.

89. The estimated calcidiol exposures from calcidiol-containing supplements range from 10 to 20 µg/day, which is equivalent to 0.14 to 0.28 µg/kg bw/day.

Total exposure estimates from food and supplements combined

90. Total exposure estimates to calcidiol from food and supplement sources combined in women aged 16-49 years are presented in Tables 6 and 7 below. For acute exposure estimates, total exposure from food sources (Table 2) was summed with exposure data from dietary supplements (Table 5). For chronic exposure estimates, total exposure from food sources (Table 4) was summed with exposure data from dietary supplements (Table 5).

91. To calculate the minimum total exposures in Tables 6 and 7, the lowest supplement exposure (10 µg/person/day or 0.14 µg/kg bw/day) was summed with the minimum exposures from food (Tables 4 and 5) for both mean and 97.5th percentile consumption. To calculate the maximum total exposures as seen in Tables 6 and 7, the highest supplement exposures from Table 5 (20 µg/person/day or 0.28 µg/kg bw/day) were summed with the maximum exposures from food (Tables 3 and 4) for both mean and 97.5th percentile consumption.

Table 6. Estimated total acute calcidiol exposure from food sources combined with supplements in women aged 16-49 years.

Total calcidiol exposure (food + supplements)	(ug/person/day)*	P97.5	(µg/kg bw/day)* Mean	P97.5
Minimum	10	11	0.14	0.15
Maximum	20	21	0.29	0.3

* Rounded to 2 s.f.

92. Minimum total acute calcidiol exposures from all dietary sources including supplements, for women aged 16-49 years, are 10 µg/day and 11 µg/day for mean and 97.5th percentile consumption, respectively. Maximum total acute exposures from all dietary sources including supplements are 20 µg/day and 21 µg/day mean and 97.5th percentile, respectively.

93. ……..

Table 7. Estimated total chronic calcidiol exposure from food sources combined with supplements in women aged 16-49 years.

Total calcidiol exposure (food + supplements)	**(ug/person/day)* Mean**	**P97.5**	**(µg/kg bw/day)* Mean**	**P97.5**
Minimum	10	10	0.14	0.14
Maximum	20	21	0.28	0.29

* Rounded to 2 s.f.

94. Minimum total chronic calcidiol exposure from all dietary sources including supplements amongst women aged 16-49 years is 10 µg/day for both mean and 97.5th percentile groups. Maximum total chronic exposures from all food sources are 20 µg/day and 21 µg/day mean and 97.5th percentile values, respectively. Exposure to calcidiol from dietary sources are minor relative to exposure from supplements.

Risk characterisation

95. All calcidiol-containing-containing supplements available on the market did not exceed the ACNFP TUL of 40 µg/day. All supplements currently available on the market with the exception of “D.velop Tablets Adult” were at the EFSA safe intake level of 10 µg/day. The “D.velop Tablets Adult” exceeded the EFSA safe intake level by 2-fold.

96. The highest estimated exposures of calcidiol from food sources only, was 1.1 µg/day, which is significantly below the ACNFP TUL of 40 µg/day and the EFSA safe intake level of 10 µg/day. For food and calcidiol supplements combined, maximum mean exposures of calcidiol exceeded the EFSA safe intake level of 10 µg/day by 2-fold. The minimum 97.5th percentile intake marginally exceeded the EFSA safe intake level, whereas the maximum 97.5th percentile intake exceeded the EFSA safe intake level by 2.1-fold. However, all mean and 97.5th percentile chronic intakes of calcidiol from food and supplements combined were below the ACNFP TUL of 40 µg/day. Individuals with a loss of function mutation in enzyme CYP24A1 are more likely to have higher circulating levels of calcidiol in their blood as this enzyme is responsible for the breakdown of calcidiol and 1,25(OH)2D (Jones et al., 2012) and are therefore more prone to the effects of excessive calcidiol exposure.

97. It should also be noted that supplements are likely to be the greatest contributor to calcidiol intake in women of childbearing age, whereas calcidiol intake from the food sources alone is low. Furthermore, not all women of child-bearing age consume supplements. Results from the most recent NDNS (years 9-11) report have shown that between 2016-2019 20% of female respondents aged 19-64 years consume vitamin D supplements (Bates et al, 2020).

98. The main uncertainties in this assessment were that the NDNS does not specifically include data for pregnant and lactating women, so women of child-bearing age (i.e. 16-49 years) were used as a proxy for these consumer groups, and there is little information on how their diets might differ.

99. Further uncertainties include the limited data available on calcidiol's safety in pregnant women.

100. Other uncertainties may include background exposure from UVB radiation. Although, exposure to UVB radiation is unlikely to result in adverse

serum 25(OH) levels, even when consuming dietary intakes of calcidiol, due to an inbuilt mechanism in the skin. SACN, 2016 stated that “prolonged sunlight exposure does not lead to excess production of cutaneous vitamin D”. This is “because endogenously produced pre-vitamin D3 and vitamin D3 are photolyzed to inert compounds” (SACN, 2018), thus preventing conversion into its primary metabolite; 25(OH)D, (i.e. calcidiol) (ACNFP, 2024; Perez-Lopez et al., 2015). SACN also stated that “Vitamin D3 is produced endogenously from 7-dehydrocholesterol (7-DHC) in the skin of humans and animals by the action of sunlight containing UVB radiation (wavelength 280-315 nm) or by artificial UVB light”. The 7-DHC in the epidermis is converted to pre-vitamin D3, which reaches a maximum concentration in the skin within a few hours (Holick et al., 1980)” (SACN, 2018).

Conclusions

101. Exposure in, pregnant and lactating women, and women attempting conception who do not take calcidiol supplements, and whose only exposure to calcidiol is from food sources, does not exceed the ACNFP TUL of 40 µg/day and EFSA’s safe intake level of 10 µg/day.

102. When considering exposure estimates from all sources (food and supplements combined), for women of childbearing age, all intakes were below the ACNFP TUL of 40 µg/day. Only the minimum and maximum 97.5th percentile intakes exceed the EFSA safe intake level of 10 µg/day up to 1.1 and 2.1-fold respectively. However, it should be noted supplements are likely the greatest contributor to calcidiol exposure in these population groups. Furthermore, not all women of child-bearing age take supplements, it has been estimated that 20% of females aged 19-64 years take vitamin D supplements.

103. Ultimately, whilst exposure in healthy pregnant and lactating women from calcidiol supplements is unlikely to exceed the established Health Based Guidance Values, sensitive individuals with loss or function mutations would be more susceptible to the effects of calcidiol.

This is a paper for discussion. This does not represent the views of the Committee and should not be cited.

Questions on which the views of the Committee are sought

1. Does the Committee have any comments on the potential risks of calcidiol supplements on maternal or fetal health?

2. Is the Committee content with using a HBGV of 10 or 40 µg/day for risk characterisation?

3. Does the Committee have any other comments on the contents of this review?

Secretariat
December 2024

This is a paper for discussion. This does not represent the views of the Committee and should not be cited.

List of Abbreviations and Technical terms

25(OH)D	25-hydroxyvitamin D
ACNFP	Advisory Committee on Novel Foods and Processes
COT	Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment
EC	European Commission
EFSA	The European Food Safety Authority
FSA	Food Standards Agency
FSS	Food Standards Scotland
HBGV	Health Based Guidance Value
IU	International Units
LOAEL	Lowest Observed Adverse Effect Level
mg	milligrams
NDA	Panel on Nutrition, Novel Foods and Food Allergens (NDA)
NDNS	National Diet and Nutrition Survey
ng	Nanograms
nmol	Nanomole
NOAEL	No Observed Adverse Effect Level
OECD	The Organization for Economic Cooperation and Development
kg	Kilograms
RCT	Randomised Clinical Trial
SACN	Scientific Advisory Committee on Nutrition
TUL	Tolerable Upper Intake Level
UL	Upper level
VDE	Vitamin D equivalents

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Search Terms

The references cited in this discussion paper are of publications found in Pubmed, Scopus and Springer, searched using Lit fetch. The publication retrieved were selected using the following search terms:

Calcidiol AND toxicity,
Calcidiol AND Pregnancy,
Calcidiol AND lactation,
Calcidiol AND conception,
Calcidiol AND birth defects,
Calcidiol AND vitamin D,
Calcidiol AND supplements,
Calcifediol AND safety,
25 (OH)D AND safety.

TOX/2024/45 Annex A

The data presented in the tables below are based on consumers of foods reported in the NDNS ((Bates et al., 2014, 2016; 2018, 2020).

Table A1: Estimated acute consumption of foods containing Calcidiol for women of childbearing age (16-49 years).

Food Groups	No. of consumers	Consumption (g/person/day) * Mean	97.5	Consumption (g/kg bw/day) * Mean	P97.5
Semi-skimmed milk	2083	200	620	3	9.7
Whole milk	1333	130	560	2	8.5
Butter	1736	16	51	0.24	0.77
Egg yolk	2128	34	93	0.5	1.4
Salmon	375	79	200	1.2	3.3
Trout	168	78	240	1.2	3.7
Pork cuts	1406	70	210	1	3.1
Pork rind	69	16	62	0.23	0.89
Pork liver	68	20	53	0.28	0.7
Cow Kidney	17**	15	27	0.21	0.43
Beef Liver	96	36	120	0.54	2.1

*Rounded to 2 s.f.

** Consumption or exposure estimates made with a small number of consumers may not be accurate. Where the number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers.

Table A2: Estimated chronic consumption of foods containing Calcidiol for women of childbearing age (16-49 years).

Food Groups	**No. of consumers**	**(g/person/day) * Mean**	**P97.5**	**(g/kg bw/day) * Mean**	**P97.5**
Semi-skimmed milk	2083	110	400	1.7	5.7
Whole milk	1333	65	330	1	5
Butter	1736	6.9	25	0.1	0.4
Egg yolk	2128	13	39	0.2	0.63
Salmon	375	22	54	0.34	0.89
Trout	168	22	73	0.3	1
Pork cuts	1406	23	81	0.34	1.3
Pork rind	69	4.3	15	0.064	0.23
Pork liver	68	5.8	19	0.084	0.27
Cow Kidney	17**	3.9	7.4	0.054	0.11
Beef Liver	96	10	35	0.15	0.54

*Rounded to 2 s.f.
** Consumption or exposure estimates made with a small number of consumers may not be accurate. Where the number of consumers is less than 60, this should be treated with caution and may not be representative for a large number of consumers.