Toxicity

In this guide

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 Calcidiol was found to not be mutagenic in both experiments (Remus and Verspeek-Rip, unpublished report, 2016).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 D3 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 D3 and 1.7 - 16,770 µg/kg bw/day, for calcidiol. Study authors reported “signs of excessive intake of vitamin D3” were observed at doses of vitamin D3 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 D3 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 D3 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 µ g/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 D3. 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 D3 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 D3 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)D3 level. Participants received either 5, 10 or 15 µg of calcidiol or 20 µg/vitamin D3 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 D3 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 D2 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 D3 (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 D3 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 D3. 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 D3 group. Overall, safety laboratory parameters remained within normal reference ranges. None of the adverse events reported were considered related to calcidiol or vitamin D3 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 D3 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 D3 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 D3 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 D3 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 D2 and D3 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).