ANSES Scientific & Technical Support
Request No 2022-AST-0099

The Director General

Maisons-Alfort, 26 October 2022

Scientific and Technical Support NOTE

of the French Agency for Food, Environmental and Occupational Health & Safety

on the application to cholecalciferol (vitamin D3) of the provisions relating to substances with endocrine-disrupting properties of Act No. 2020-105 of 10 February 2020 (the "AGEC Act")[1]

On 30 May 2022, ANSES received a formal request from the Directorate General for Health (DGS) and the Directorate General for Risk Prevention (DGPR) to provide scientific and technical support for the following: Formal request relating to cholecalciferol (vitamin D3) in connection with implementation of the provisions of Article 13-II of Act No. 2020-105 of 10 February 2020 on waste reduction and the circular economy (the "AGEC Act"), concerning the availability of public information enabling the identification of endocrine disruptors (EDs) in products.

1. BACKGROUND AND PURPOSE OF THE REQUEST

Two ministerial orders are planned in connection with Article 13-II of the AGEC Act and its implementing decree no. 2021-1110 of 23 August 2021 on the availability of information enabling the identification of endocrine disruptors in products, and on the basis of the Agency's opinion of 25 March 2021 (ANSES 2021e):

- one (required by Article R. 5232-19 of the Public Health Code) establishing, following a proposal from ANSES, the list of substances with endocrine-disrupting properties classified according to the level of scientific evidence as known, presumed or suspected, and the list of product categories with a particular risk of exposure;
- the other (required by Article R. 5232-20 of the Public Health Code) specifying the conditions relating to the content and presentation of information.

[1] Cancels and replaces the version dated 29 September 2022. The modification details are provided in the table in Annexe 1.

The draft order establishing the list of substances with endocrine-disrupting properties includes those identified as substances of very high concern (SVHC) for their endocrine-disrupting effects under Regulation (EU) No 1907/2006 (the REACH Regulation), as well as the biocidal (including cholecalciferol) and plant protection active substances identified as EDs during assessment of the dossiers submitted for their authorisation.

Regarding cholecalciferol (or vitamin D3; CAS no. 67-97-0), its characterisation as an ED was established during its assessment under the Biocides Regulation (EU) No 528/2012 for use as a rodenticide. In this context, because it fulfilled the identification criteria mentioned in Regulation (EU) No 2017/2100[2], cholecalciferol was added to the list of substances identified as EDs at European level and published on the EDlists.org website (List I), which explains its inclusion in the annex of the draft order. This approach is consistent with the ranking principles associated with the list of substances of interest published by ANSES (ANSES 2021c), which encourage the pooling of results from different fields, in view of the recent nature of work on assessing the ED hazard of substances.

In another regulatory context, vitamin D is included in Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods, and more specifically in its Annex I on vitamins and minerals which may be added to foods. In a regulatory sense, this annex constitutes a positive list.

ANSES was therefore asked to clarify the properties of vitamin D in terms of both its nutritional benefits and the health risks it may entail, especially those relating to its action on the endocrine system. This note aims at providing a summary of the scientific knowledge available to date to the ministries involved, enabling them to enact the orders outlined above in the interests of public health.

2. ORGANISATION OF THE WORK

The toxicological profile of cholecalciferol is based on the European assessment report drawn up under the Biocides Regulation for its use as a rodenticide[3] and from which summaries or extracts have been taken; if further clarification is needed, ANSES invites the reader to refer to this report.

The reminders on the concept of endocrine disruption are based on the work of two expert rapporteurs, members of the "Endocrine Disruptors" working group set up by ANSES and reporting to the Expert Committee on "Chemicals covered by the REACH and CLP[4] regulations". More broadly, reference has also been made to the opinions issued by these same ANSES groups on the scientific criteria defining EDs (ANSES 2016a, 2021c, d), or those of other agencies.

The summary on the nutritional benefits and risks of cholecalciferol is based on work previously carried out by EFSA and ANSES.

In addition, the Agency interviewed Professor Jean-Claude Souberbielle, a specialist renowned for his work on vitamin D, in order to gather the most up-to-date scientific information on its metabolism, mechanisms of action and nutritional value. Professor Souberbielle had, on

[2] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R2100&from=EN
[3] At biocidal doses, cholecalciferol causes hypercalcaemia resulting in the death of rodents within 3 to 10 days of exposure
[4] Regulation (EU) No 1272/2008 on classification, labelling and packaging of substances and mixtures

behalf of a group of experts representing various scientific organisations, contacted both the Agency and the Ministry of Health at the time of dissemination of the above-mentioned draft order, putting himself at their disposal.

ANSES also consulted various agencies in the Agency network facilitation committee (CASA) on a draft version of this scientific and technical support note. The responses received were taken into account[5].

Moreover, although this was not an expert appraisal in health risk assessment but an examination of existing knowledge, ANSES decided to formally consult the experts of the Expert Committee on "Human Nutrition" (2018-2022 mandate) and the Permanent Working Group on "Endocrine Disruptors", on the basis of the scientific information (excluding the conclusions) in the post-consultation version. The comments received were also taken into account.

After outlining the toxicological profile of cholecalciferol, pointing out its potentially harmful effects through its action on the endocrine system, this note will provide a reminder of what EDs are. Lastly, it will review what is known about the health benefits of cholecalciferol, as well as the risks of inadequate intake or, on the contrary, accidental overdose.

Besides cholecalciferol, the note will discuss similar nutrients that could potentially interfere with the endocrine system through their mechanisms of action.

3. ANALYSIS AND CONCLUSIONS

3.1. Toxicological profile of cholecalciferol

The toxicological profile presented below is based on the European assessment report drawn up in connection with the inclusion of cholecalciferol in Annex I of Regulation (EU) No 528/2012 concerning the making available on the market and use of biocidal products[6], for its use as a rodenticide (ECHA 2018). This report presents the assessment of the applicant's dossier by Sweden, which was appointed as rapporteur Member State for this substance. As required by the Biocides Regulation, this report was used to produce the opinion of the Biocidal Products Committee of the European Chemicals Agency (ECHA) prior to the decision to authorise the substance (ECHA 2017).

3.1.1. Toxicokinetics

The abundance of data available for humans enabled the following toxicokinetic characteristics to be established.

Cholecalciferol is rapidly absorbed from the intestines, although there is considerable inter-individual variability (absorption rates between 55% and 99% in healthy subjects). It is metabolised by the liver into 25-hydroxycholecalciferol (25(OH)D or calcidiol), which is then transported by a plasma protein, vitamin D-binding protein (DBP), to the kidneys, where it is

[5] Responses were received from the French National Agency for Medicines and Health Products Safety (ANSM), *Santé Publique France* (SpF), the High Council for Public Health (HCSP) and the National Cancer Institute (INCa)

[6] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32012R0528&from=EN

further metabolised into 1,25-dihydroxycholecalciferol (1,25$(OH)_2$D or calcitriol), which is the biologically active form of vitamin D.

Because of its lipophilicity, cholecalciferol tends to be distributed in adipose tissue. Its plasma half-life is 4-5 days, while that of 25(OH)D is 15-30 days and that of 1,25$(OH)_2$D is about 14 hours.

Cholecalciferol metabolites are mainly excreted in faeces via bile, and to a lesser extent in urine.

3.1.2. Acute toxicity

The oral LD50[7] values observed in rats are 35 mg/kg in males and 47 mg/kg in females. The main acute effects observed are neurotoxic, potentially as a result of hypercalcaemia, given the hypercalcinosis observed at autopsy in various organs (heart, spleen, kidneys and blood vessels) from the dose of 25 mg/kg.

3.1.3. Repeated dose toxicity

According to the human data identified by EFSA, hypercalcaemia is the critical effect on which the NOAEL[8] of 4.2 µg/kg bw/day is based.

In rats, hypercalcaemia co-occurred with renal and adrenal changes, which were observed in 28-day and 90-day oral studies. A slight increase in blood calcium levels (+4%) was observed in the 90-day study from the dose of 0.06 mg/kg bw/day. Given the small magnitude of this increase, this dose is considered more of a NOAEL than a LOAEL[9]. However, considering that renal damage (tubular degeneration and dilatation) was observed at this dose, the assessment report did not rule out the possibility that the NOAEL could be reduced to 0.012 mg/kg bw/day (which is still three times the NOAEL identified in humans).

3.1.4. Genotoxicity

As a reminder, the testing strategy recommended by EFSA for assessing the genotoxicity of food substances is broken down into several steps (EFSA 2011). The first step is based on two *in vitro* tests: an Ames test and a micronucleus assay. If neither are positive, the substance is considered to be non-genotoxic. If either of these are equivocal, further *in vitro* tests are needed to clarify the overall result. If one of the tests is clearly positive, *in vivo* tests are required. In the case of cholecalciferol, three types of *in vitro* tests were conducted (Ames tests, mammalian cell gene mutation test and chromosomal aberration test). One of the Ames tests was positive. An *in vivo* study combining a comet assay and a micronucleus assay was negative on the "micronucleus" component (with a doubt about exposure of the bone marrow cells tested) and positive on the "comet" component studied on hepatocytes. In view of all these data, ECHA's Committee for Risk Assessment (RAC) considered that **cholecalciferol, taking the weight of evidence into account, does not meet the criteria for mutagenicity as defined in the CLP Regulation**.

[7] LD50: Median lethal dose killing 50% of animals within 14 days of a single administration
[8] No observed adverse effect level: maximum dose not leading to statistically significant harmful biological or health effects in comparison with the control, from the identification of the LOAEL. In other words, it is the tested dose that directly precedes the LOAEL.
[9] Lowest observed adverse effect level: minimum dose leading to an observed harmful effect.

3.1.5.Carcinogenicity

No carcinogenesis studies are available that can be used to analyse the effect of supraphysiological doses on cell proliferation over the entire lifetime of animals. On the other hand, in a 26-week study, rats exposed to 0.25 mg/kg bw/day developed hyperplastic adrenal nodules and pheochromocytomas. These observations led to a NOAEL of 0.125 mg/kg bw/day being identified. However, the occurrence of neoplastic effects at this dose cannot be ruled out beyond 26 weeks of exposure. In view of this evidence, the Committee for Risk Assessment concluded in December 2016 that **cholecalciferol should not be classified as carcinogenic**.

3.1.6.Fertility and developmental toxicity

Data on fertility and developmental toxicology are scarce, and inadequate for exploring the reprotoxic potential of cholecalciferol. The applicant for inclusion of cholecalciferol in Annex I of the Biocides Regulation argued for a waiver from testing, on the basis of the abundance of data on human populations supporting the absence of adverse effects of vitamin D3 on human reproductive health and development up to the highest dose studied during pregnancy (100 µg/day). The applicant also argued that biocidal use of cholecalciferol only marginally contributes to total vitamin D exposure. In view of this evidence, the Committee for Risk Assessment concluded in December 2016 that **cholecalciferol should not be classified as toxic to reproduction**.

3.1.7.Neurotoxicity

There are no studies specifically targeting neurotoxicity. On the other hand, some functional effects have been observed in rats exposed for 90 days to the highest dose (0.06 mg/kg bw/day): decreases in landing foot splay and grip strength in males, and decreased motor activity in females. However, these effects each concerned only one of the two sexes. Moreover, considering that they were not associated with histological lesions of the nervous tissues and occurred at doses marked by hypercalcaemia and organ calcification, **the effects observed are considered to reflect general toxicity rather than toxicity specifically targeting the nervous system**.

3.1.8. Endocrine-disrupting properties

The "biocides" assessment report considers cholecalciferol to be a prohormone, whose active metabolite plays a major role in maintaining calcium and phosphorus homeostasis. It also induces hypercalcaemia and tissue calcification in rats. The report therefore considers that **cholecalciferol fulfils the criteria in sections A and B of the Annex to Regulation (EU) No 2017/2100 setting out scientific criteria for the determination of ED properties, within the meaning of the Biocides Regulation**.

3.2. Endocrine disruptors

3.2.1.Definitions

Since the declaration at the Wingspread conference in 1991[10], which set out the concept of endocrine disruptors, several definitions of EDs have been developed. The definition given by the World Health Organization in 2002 is the one most commonly accepted: *"An endocrine disruptor (ED) is an* ***exogenous*** *substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations".*

For the Ministry of Health and Prevention, "*an endocrine disruptor (ED) is a chemical of natural or synthetic origin,* ***a foreign substance to the body*** *liable to interfere with the functioning of the* ***endocrine system****, i.e. the cells and organs involved in the production of hormones and their action on "target" cells via receptors. EDs disrupt the hormonal functioning of living organisms, and thus have* ***adverse effects*** *on the environment and human health."*[11]

The concept of "foreign" goes beyond "exogenous" as it includes all xenobiotics, thus excluding from this definition of EDs any exogenous substance with a physiological role, such as nutrients.

Like all endocrine active substances or EASs as defined by EFSA (EFSA 2013), EDs can act schematically at three levels (Gore *et al.* 2015, La Merrill *et al.* 2020). They can therefore:

- modify hormone production or secretion by acting on the endocrine cells;
- affect a hormone's bioavailability by acting on its metabolism or plasma transport proteins;
- act on target cells and exert hormone agonist or antagonist activity by binding to this hormone's receptors or altering its signalling pathways.

The challenge of a scientific assessment aiming to determine substances meeting the WHO's definition of EDs is to identify which of the EASs lead to a harmful effect due to their activity on the endocrine system (ANSES 2021c).

Furthermore, a characteristic of some substances identified as EDs is that they may have effects of concern at low doses, distinct from the effects observed at higher doses in conventional toxicology studies (Vandenberg *et al.* 2012). It may therefore be inappropriate to assess the risks of such substances using conventional methods, unless scientific data on the shape of the exposure/effect relationship are available.

This had contributed to the establishment of ANSES's position through different opinions (ANSES 2016a, 2021d) to recommend establishing the ED nature as a hazard class, mainly in the framework of the CLP Regulation, and to recommend adapted management in various sectoral regulations according to uses and populations (e.g. the regulation on toys).

3.2.2.Endocrinology

All these definitions refer to "the endocrine system" or "endocrinology" or "hormones".

[10] https://www.senat.fr/rap/r10-765/r10-76514.html
[11] https://solidarites-sante.gouv.fr/sante-et-environnement/risques-microbiologiques-physiques-et-chimiques/article/perturbateurs-endocriniens#:~:text=Un%20Perturbateur%20Endocrinien%20(PE)%20est,impliqu%C3%A9s%20dans%20la%20production%20des

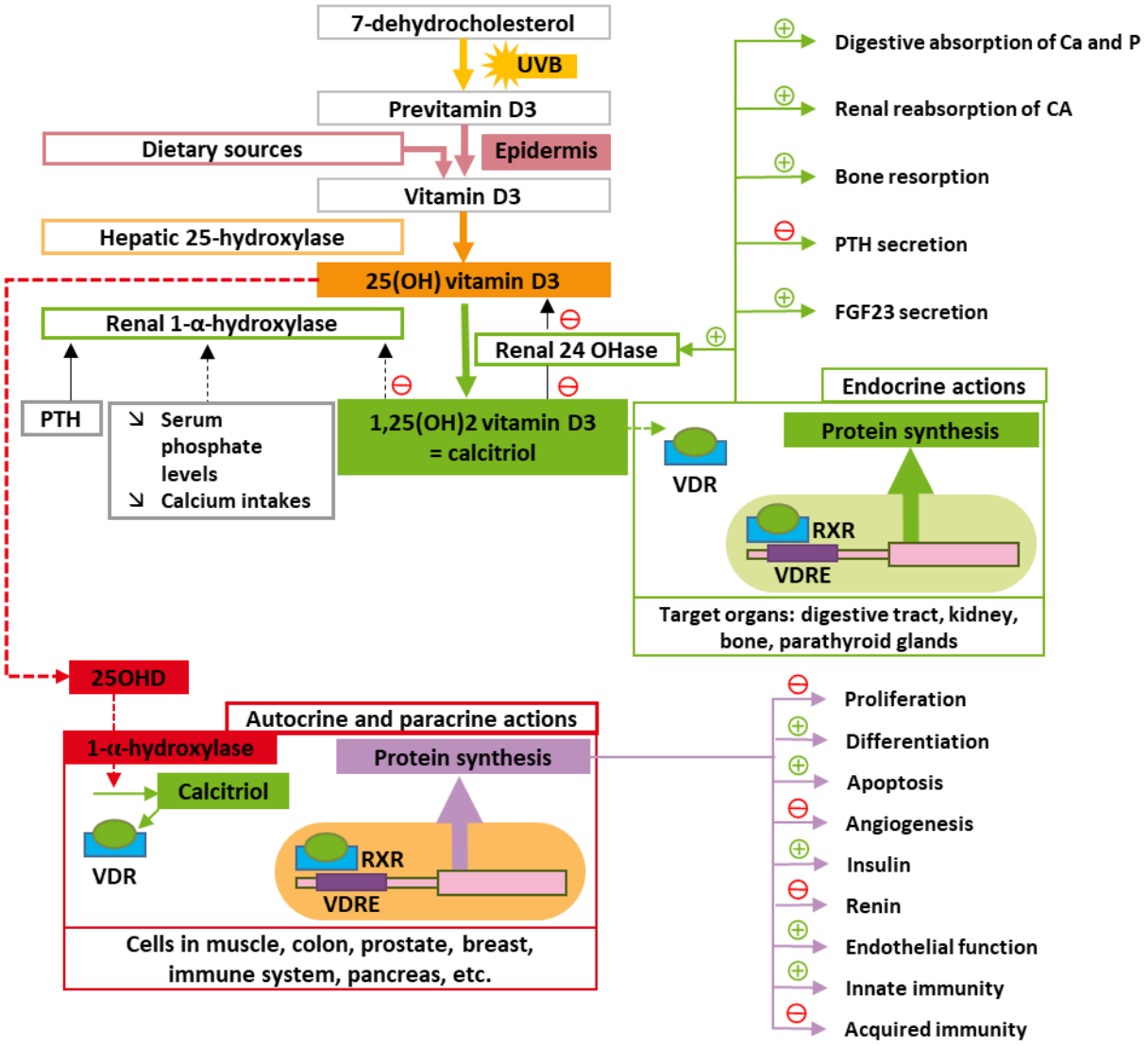
In the historical definition, a hormone is a substance produced by endocrine cells, which acts at a distance via the bloodstream on other "target cells" whose development and/or functions it controls. The hormone acts on a target cell by binding to specific hormone receptors. Physiological regulatory systems constantly adjust hormone secretion to meet the body's needs.

This principle is nowadays qualified by two commonly accepted ideas:

- endocrine cells are not necessarily grouped together in an organ called an "endocrine gland";
- hormones do not always travel to distant target cells via the bloodstream. Indeed, a hormone can act within the organ that produced it. This extension of endocrinology in the strict sense (transport of messengers through the bloodstream) to paracrinology and autocrinology[12] (diffusion within the organ) therefore considerably opens up the disciplinary field of endocrinology and, consequently, of endocrine disruptors.

In the case of cholecalciferol, the active hormone (calcitriol) is produced in many organs, where it diffuses and acts (Figure 1).

[12] Paracrine regulation occurs when the hormone acts on a different cell type from the one that secreted it, after simple diffusion in the interstitial fluid of the organ (e.g. testosterone produced by Leydig cells acts on neighbouring Sertoli cells). Autocrine regulation occurs when a cell is itself the target of the hormone it secretes (e.g. granulosa cells of ovarian follicles).



Abbreviations used in the figure: FGF23 (fibroblast growth factor 23), PTH (parathormone), RXR (retinoic acid receptor), VDR (Vitamin D receptor), VDRE (Vitamin D-response element)

Figure 1: Pathways by which vitamin D regulates calcium and phosphorus metabolism (Courbebaisse and Souberbielle 2011)

3.2.3. General ED identification criteria and the case of the Biocides Regulation

In line with the WHO definition, for a substance to be considered an ED, it must meet three criteria:

1) it must cause adverse effects on health (human health component) or on wildlife populations (environmental component);
2) it must interact with the endocrine system;
3) this interaction must be the cause of the observed adverse effects.

These criteria are also those adopted by ANSES in its opinion defining a methodology for assessing the endocrine-disrupting nature of chemicals (ANSES 2021c) and are derived largely from the joint JRC/EFSA/ECHA work.

From a regulatory point of view, these criteria were defined by the European Commission on 15 June 2016 and then rejected by the European Parliament in 2017, before finally being adopted by the European Commission on 19 April 2018 in Regulation (EU) No 2018/605[13] and Delegated Regulation (EU) No 2017/2100, which respectively define the criteria for identifying a plant protection and a biocidal active substance as an ED. Pursuant to Regulation (EU) 528/2012 on biocidal products, Delegated Regulation (EU) 2017/2100 of 4 September 2017 includes these criteria in its annex, setting them out for substances disrupting the endocrine system of humans (part A) or of non-target organisms of biocidal products (part B).

In this context, cholecalciferol has been assessed as meeting all three criteria since it can cause hypercalcaemia with potentially harmful consequences in humans and rats (criterion 1); it interacts with the endocrine system by behaving as a preprohormone, leading to the formation of calcitriol, which is itself a hormone that binds to distant receptors and regulates the production of other hormones such as parathormone whose secretion it inhibits (criterion 2); the hypercalcaemia results from its action on the endocrine system (criterion 3)

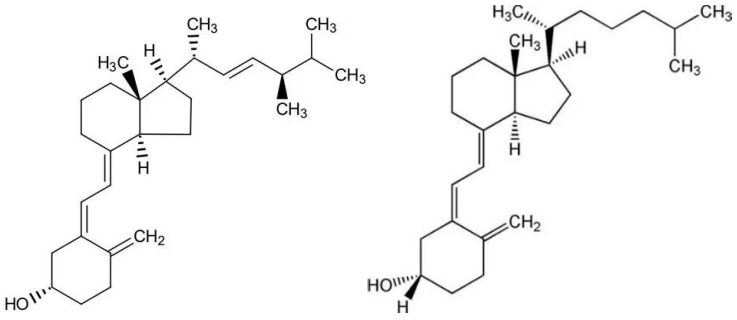
However, under the regulations on biocidal active substances, these criteria apply to all active substances, regardless of whether they are endogenous or "foreign".

In view of this evidence, it appears that **application to cholecalciferol of the identification criteria set out in Regulation (EU) No 2017/2100 leads to it being identified as a biocidal active substance that meets the definition of EDs** (ANSES 2021b).

3.3. Benefits and risks of cholecalciferol

3.3.1. General points

Vitamin D refers to two compounds: ergocalciferol (or vitamin D2), which is synthesised by plants, and cholecalciferol (or vitamin D3), which is synthesised by animals including humans (Figure 2). Both follow the same metabolic pathways leading to equivalent biological activity.



Ergocalciferol (vitamin D2) Cholecalciferol (vitamin D3)

Figure 2: Molecular formulas of ergocalciferol and cholecalciferol

Cholecalciferol, whether dietary or derived from dermal synthesis, is hydroxylated in the liver to 25-hydroxycholecalciferol (25(OH)D or calcidiol), which is the reserve form in the body. This is transported to the kidneys where it is hydroxylated again to 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$ or calcitriol), which is the biologically active form.

[13] Regulation (EU) 2018/605 of 19 April 2018 amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine-disrupting properties https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02018R0605-20180420

Dietary cholecalciferol comes from sources such as oily fish (smoked herring contains 22 µg/100 g and sardines contain 14 µg/100 g)[14], offal (calf's liver contains 2.52 µg/100 g) and egg yolk (2.11 µg/100 g), to name but a few. In addition to cholecalciferol, these foods also provide small amounts of 25(OH)D (Schmid and Walther 2013).

Cholecalciferol is synthesised in the skin by the deep cells of the epidermis under the action of ultraviolet B (UVB) radiation. The intensity of this synthesis depends on the latitude where a person lives, the season, the person's age, skin pigmentation and whether or not the skin is screened (by clothing or sun cream).

3.3.2.Benefits of vitamin D

Vitamin D contributes mainly to maintaining calcium and phosphorus homeostasis, with the help of parathyroid hormone, also called parathormone (PTH), and ensures the mineralisation of certain tissues (bones, cartilage and teeth) during and after growth (ANSES 2021a). Figure 1 summarises the main pathways by which vitamin D regulates calcium and phosphorus metabolism.

In people with inadequate vitamin D status, daily vitamin D supplementation at doses of 20 µg/day (800 IU/day) has been shown to reduce the risk of peripheral fractures (such as femoral neck fractures) (Bischoff-Ferrari *et al.* 2012) and falls (Bischoff-Ferrari *et al.* 2019), particularly when combined with adequate calcium intake (Weaver *et al.* 2016). In addition to its role in maintaining the calcium and phosphorus balance essential for mineralised tissues, vitamin D appears to participate in the immune system response by reducing the risk of respiratory infections (Martineau *et al.* 2017). It may also play a role in metabolic balance by reducing the risk of pre-eclampsia in pregnant women (Palacios, Kostiuk and Peña-Rosas 2019), and blood pressure (modestly but significantly) in hypertensive and vitamin D-deficient individuals (Chen 2022). According to some authors, vitamin D supplementation may also improve the life expectancy of cancer patients (Keum *et al.* 2022)[15]. Vitamin D helps reduce the risk of developing autoimmune diseases (Hahn *et al.* 2022). Its neuroprotective effect is also mentioned (Cortés-Albornoz *et al.* 2021).

In summary, these studies (of which only a few references are mentioned in this note, which does not claim to be exhaustive) suggest a benefit from a daily intake of vitamin D to meet requirements, but also health risks in the event of a deficiency. ANSES nevertheless points out that all sources of intake should be taken into account: endogenous production and dietary intake through appropriate choices, as well as supplementation or consumption of fortified foods if the first two sources are inadequate.

Based on the assumption of zero endogenous synthesis, although this cannot be quantified given its great variability (depending in particular on the seasons, geographical location, individual characteristics, etc.), a value of 15 µg cholecalciferol/day was adopted by EFSA in 2016 as an adequate intake, enabling both men and women from 1 year of age onwards to reach the serum 25(OH)D concentration threshold of 50 nmol/L (or 20 ng/mL), considered adequate. This value was endorsed by ANSES in March 2021 as a nutritional reference value (ANSES 2021b). Given the wide variability in vitamin D requirements depending on individual

[14] Levels according to the Ciqual food composition tables (https://ciqual.anses.fr/)
[15] This benefit is currently controversial in view of the report by the National Cancer Institute (INCa) on the impact of nutritional factors during and after cancer, which emphasises that vitamin D is not a conclusive nutritional factor for making recommendations to cancer patients (INCa 2020).

characteristics (particularly age and skin colour), time spent outdoors and the latitude where a person lives, an individualised approach to meeting the requirements would be better.

Particular attention has been paid to meeting the requirements of children, and led to the introduction of systematic supplementation of children to prevent rickets. Recommendations have been developed by the French Paediatric Society (Vidailhet *et al.* 2012) and were recently updated by representatives of various scientific organisations[16], leading to the recommendation to supplement children from 0 to 18 years of age with vitamin D doses of between 400 and 800 IU[17] per day or, failing that, to supplement children from 2 years of age onwards with four annual doses of 50,000 IU or two annual doses of 80,000 to 100,000 IU (Bacchetta *et al.* 2022).

3.3.3.Risks associated with cholecalciferol overdose

In the event of excessive cholecalciferol intake, the excess production of $1,25(OH)_2D$ induces an increase in blood calcium levels that activates the kidney enzyme 24-hydroxylase (Figure 1). This converts 25(OH)D and $1,25(OH)_2D$ into the inactive metabolites $24,25(OH)_2D$ and $1,24,25(OH)_3D$ respectively (Jones, Prosser and Kaufmann 2012). This regulatory mechanism buffers the fluctuations in blood calcium levels mainly induced by the change in 25(OH)D concentration. Individuals with a mutation in the gene coding for 24-hydroxylase, and therefore lacking this mechanism, are at increased risk of developing hypercalcaemia and complications such as nephrocalcinosis in the first few months of life (Azer *et al.* 2021).

However, this regulatory mechanism has its limits in the event of acute poisoning, as it takes several weeks to develop (Wagner *et al.* 2011).

Symptoms associated with hypercalcaemia vary and may include fatigue, muscle weakness, anorexia, nausea, vomiting, constipation, heart rhythm disorders, soft tissue calcification and weight loss. It is sometimes accompanied by hypercalciuria. Sustained hypercalcaemia can lead to nephrolithiasis, and even nephrocalcinosis and kidney failure (EFSA 2012).

In 2012, EFSA published an opinion setting a tolerable upper intake level (UL)[18] of vitamin D for adults, based on the absence of hypercalcaemia in two studies of 10 and 15 healthy men exposed to vitamin D doses of 234-275 µg/day for periods ranging from 2 to 5 months (Barger-Lux *et al.* 1998, Heaney *et al.* 2003). Considering that these studies identified a NOAEL of 250 µg/day per individual and applying an uncertainty factor of 2.5 to this NOAEL, EFSA selected a UL of 100 µg/day for adults, which is also applicable to pregnant women in the absence of any evidence that they are more sensitive to the adverse effects of vitamin D.

For children under one year of age, EFSA adopted the UL of 25 µg/day set by the Scientific Committee on Food (SCF) in 2003, in the absence of any new published data that would justify the reconsideration of this value.

For children over 1 year of age, EFSA relied on two studies conducted in children aged 10 to 17 years, showing that exposure up to 50 µg/day did not induce hypercalcaemia (Fuleihan *et al.* 2006, Maalouf *et al.* 2008). In the absence of studies conducted at higher doses, or

[16] French Society of Paediatrics, French Society of Neonatology, French Society of Paediatric Endocrinology, French Society of Paediatric Rheumatology, French Society of Paediatric Nephrology, French Society of Paediatric Gastroenterology, Hepatology and Nutrition, among others
[17] As a reminder, 1 µg = 40 IU
[18] A tolerable upper intake level is the chronic maximum daily intake of a vitamin or a mineral considered unlikely to present a risk of adverse health effects for the entire population (ANSES 2016b)

evidence suggesting that children are less tolerant of vitamin D than adults, EFSA set the UL at 50 µg/day for children aged 1-10 years and 100 µg/day for children aged 11-17 years.

All these UL values were included in the EFSA summary document published in 2018 collating all the established ULs (EFSA 2018), which was endorsed by ANSES in its March 2021 opinion on the updating of the dietary reference values for the French population (ANSES 2021b). Work to update the ULs of various vitamins (including vitamin D) and minerals is currently being carried out by EFSA and is expected to be completed in 2023.

Moreover, through its nutrivigilance scheme, ANSES was informed of three cases of severe hypercalcaemia in infants aged between 2 and 3 months, which occurred after accidental administration of massive doses of vitamin D in food supplements. These doses were 30 to 40 times the UL of 25 µg/day (30,000 IU/day or 750 µg/day for one, and 40,000 IU/day or 1000 µg/day for the other two) and were administered for 40 to 55 days. Presented with a clinical picture combining a decrease in appetite and weight loss, the children were hospitalised for tests, which revealed severe hypercalcaemia (4.83 mmol/L, 3.08 mmol/L and 5.05 mmol/L respectively, whereas normal values are usually between 2.2 and 2.6 mmol/L). Two of them had nephrocalcinosis. These cases were the subject of an ANSES opinion dated 28 July 2021 (ANSES 2021a). One of these cases was also the subject of a publication (Gérard *et al.* 2022). In this context, ANSES joined forces with the French Health Products Safety Agency (ANSM), paediatric scientific societies, the National College of Midwives and French poison control centres to alert healthcare professionals and parents to the risk of overdose associated with giving vitamin D supplements to children, especially infants[19]. This vigilance is especially needed because of the importance of meeting the vitamin D requirements for this age group, which leads to virtually systematic supplementation.

In summary, inadequate cholecalciferol intake poses known risks to human health, particularly during growth. Dietary intake or endogenous synthesis stimulated by sun exposure is therefore necessary to avoid these risks. However, although there is a mechanism for regulating blood calcium levels to limit fluctuations according to the amount of cholecalciferol ingested or produced, this mechanism can be saturated by excess cholecalciferol. This disruption then leads to situations of hypercalcaemia. Daily limits (tolerable upper intake levels (ULs)) have been established to prevent the risk of intakes that could lead to hypercalcaemia.

3.4. Agency conclusions and recommendations

Cholecalciferol (vitamin D3) is partly produced endogenously, associated with exposure to the sun, and is also provided by foods of animal origin. Vitamin D (D2 or D3) is a preprohormone whose metabolism by the body produces an active hormone. Its main physiological function is to regulate calcium and phosphorus homeostasis. It also plays a role in reducing the risk of numerous diseases that could occur in people with slight or serious deficiencies. Its intake is beneficial to human health, within the limits (ULs) established to prevent the risk of hypercalcaemia.

ANSES points out that Regulation (EC) No 1925/2006 authorises vitamin D to be added to food, with values contributing to the meeting of daily requirements while remaining below the UL.

[19] https://www.anses.fr/fr/system/files/Note_complementaire_VitamineD.pdf

Because of its nature as a preprohormone and its role in the regulation of calcium and phosphorus metabolism, vitamin D has endocrine activity. It is above all an essential substance for humans, making vitamin D status a key determinant of health status. The risks of inadequate intake, especially among people who are rarely exposed to the sun, are well documented and constitute a major public health concern in France (the National Nutrition & Health Plan – PNNS) and throughout the world (WHO). Inadequate intake can lead to a deficiency with severe risks to human health, particularly during growth. This has led public health agencies and stakeholders (ANSES, EFSA, WHO, etc.) to define dietary reference values for vitamin D. In addition, recommendations for systematic supplementation of children have been formulated by scientific organisations in order to prevent rickets during the entire growth and bone mineralisation phase.

On the other hand, an excessive intake of cholecalciferol, above the established tolerable upper intake level (UL), saturates the cholecalciferol regulation mechanisms (mainly ensured by 24-hydroxylase) and leads to situations of hypercalcaemia. These have unfortunately been observed on several occasions in certain nutrivigilance cases. The extent of cholecalciferol-induced hypercalcaemia depends on the amount of this hormone resulting from dietary intake and from endogenous synthesis stimulated by sun exposure. The harmful effect observed in humans in the event of excessive intake is a direct result of its endocrine mode of action. An excess of cholecalciferol therefore clearly meets the three criteria (1 – adverse effect observed, 2 – interaction with the endocrine system, 3 – responsibility of this interaction in the onset of the adverse effect) that led ECHA's Biocidal Products Committee to classify it as an endocrine disruptor.

However, with regard to these criteria, ANSES would like to stress two specific points about vitamin D:

- The exposure/effect relationship for humans is characterised by a health benefit, at least at the dose corresponding to the adequate intake (15 µg/day), and by risks at doses above the UL (100 µg/day). The harmful effect resulting from the exceeded homeostatic regulatory capacities therefore occurs at a very high dose level, far above that obtained from food.
- Cholecalciferol is a substance that is partly derived from endogenous synthesis in the body. However, the WHO's definition of EDs relates to exogenous substances or mixtures; a point that has not been mentioned in the sectoral regulation on biocides.

While the inclusion of cholecalciferol in the list of proven EDs may seem justified – given its eligibility for the three identification criteria at high doses – in order to regulate uses such as that associated with biocidal substances, the two specificities mentioned above raise two questions, which ANSES alone cannot answer:

- In the absence of a cross-sectoral regulatory provision, such as a definition of the hazard class under the CLP Regulation, is the exogenous/endogenous nature of the substance an exclusion criterion that could vary from one regulation (food, its ingredients, additives, nutrients) to another (chemicals used in biocides)? ANSES notes that in terms of scientific risk assessment, this is contrary to the idea of "One substance, one assessment"[20], which ANSES supports and shares. The Agency

[20] ANSES supports the "One substance, one assessment" principle for the consistency and pooling of scientific expert appraisal work on substances beyond the regulatory frameworks in which they are addressed, promoted by the European Commission as part of the Green Deal

recommends standardising the way the exogenous/endogenous nature is taken into account in the regulations.

- Is the identification of a substance's ED status on labels (or equivalent accompanying provisions) a management measure to inform consumers with a view to attracting their attention, informing them or even inducing them to adopt avoidance behaviour?

Under the AGEC Act, identifying cholecalciferol as an ED on labels (or equivalent information provisions) of food products containing it is likely to provide erroneous information on the risk[21], insofar as daily intakes of vitamin D associated with food consumption are below the tolerable upper intake level defined for cholecalciferol and, moreover, avoiding these products would exacerbate the situation of requirements not being adequately met that already concerns a significant proportion of the population (34.5% of the population had an inadequate vitamin D status in 2015), as pointed out by the HCSP in its report of 21 June 2022 recommending the consumption of foods rich in vitamin D (HCSP 2022).

Combining consumption recommendations with the labelling of cholecalciferol as an ED (in the same way as for other known EDs such as bisphenol A) could potentially lead to a lower perception of the risks associated with these substances, and thus an increase in the corresponding public exposure.

The Agency also draws attention to the fact that other nutrient substances – such as iodine, for example – can potentially have harmful effects by disrupting the endocrine system at high doses, whereas they are beneficial to human health at lower doses. This calls for a response for this type of substance that is not confined to vitamin D.

Lastly, the Agency reiterates the importance for healthcare professionals to communicate on both the benefits of a diet that helps meet nutritional requirements in vitamin D and of physical activity in the open air, and the risks associated with overdose induced by a misuse of food supplements, in particular among young children.

Roger Genet

[21] Even if it were correct on characterisation of the hazard

KEY WORDS

Cholécalciférol, ergocalciférol, vitamine D, hypercalcémie, perturbateur endocrinien, système endocrinien

Cholecalciferol, ergocalciferol, vitamin D, hypercalcaemia, endocrine disruptor, endocrine system

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ANNEXE 1

Modification made to the revised version dated 29 September 2022

Page number	Modification
9	The text “In view of this evidence, it appears that application to cholecalciferol of the identification criteria set out in Regulation (EU) No 2017/2100 leads to it being identified as a biocidal active substance” has been modified with the addition of “**that meets the definition of EDs**”.