CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:
Colecalciferol, Vitamin D3

EC Number: **200-673-2**

CAS Number: **67-97-0**

Index Number: **603-180-00-4**

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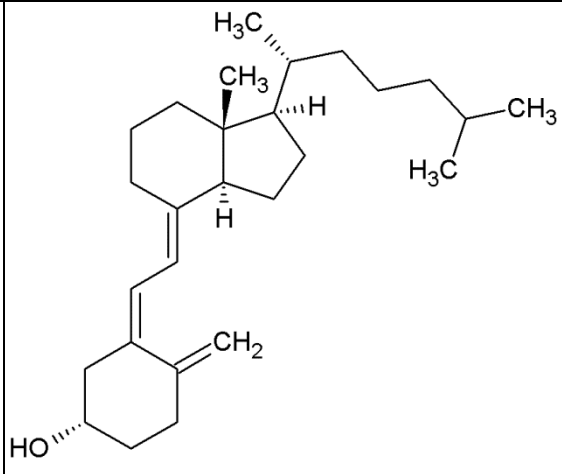
1. IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	(5Z,7E)-(3S)-9,10-secocholesta-5,7,10(19)-trien-3-ol
Other names (usual name, trade name, abbreviation)	Vitamin D3 (synonym) Colecalciferol (synonym) Cholecalciferolum (synonym, Ph. Eur.* 7.0)
ISO common name (if available and appropriate)	Cholecalciferol (ISO common name provisionally approved)
EC number (if available and appropriate)	200-673-2
EC name (if available and appropriate)	colecalciferol
CAS number (if available)	67-97-0
Other identity code (if available)	*CLP index #: 603-180-00-4*
Molecular formula	$C_{27}H_{44}O$
Structural formula	
SMILES notation (if available)	
Molecular weight or molecular weight range	384.64
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	

Description of the manufacturing process and identity of the source (for UVCB substances only)	
Degree of purity (%) (if relevant for the entry in Annex VI)	

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Cholecalciferol	Min. 97%	Acute Tox. 3*, H301 Acute Tox. 3*, H311 Acute Tox. 2*, H330 STOT RE 1, H372** GHS06 GHS08 Dgr	There are currently 20 aggregated notifications, comprising approximately 1 000 notifiers, of which 13 have the current CLH classification, accounting for the majority of notifiers (ca. 880). In addition to this, a few notifications state: -a higher oral acute toxicity classification i.e Acute Tox. 2 H300 -a higher dermal acute toxicity classification i.e. Acute Tox 2 H310 - Skin Sens. 1 H317 (one notifier) - Repr. 1B H360 and Lact. H362 (one notifier) -Aquatic Chronic 4 H413 (one notifier)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
None				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
None					

Table 5 Test substances (non-confidential information)

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**
Test Material: Vitamin D3 Vehicle: Corn oil No batch or purity stated	Not stated		A6.1.1/01
Vitamin D3 (Batch No. 520001-317-205) in corn-oil	Not stated		A6.1.1/02
Vitamin D3 (Batch No. WAD234350).	Not stated		A6.1.2/01
Vitamin D3 Batch no.: FCA83F02A	Purity 98.6%		A6.1.3
Lot/Batch number D0074	100.1%		A6.4.1/01, A6.6.2, A6.6.3, A6.6.4 (90 day study, rat, genotoxicity/mutagenicity studies)
Not stated in report	≥ 97%		A6.6.1/02 (genotoxicity/mutagenicity studies)
Vitamin D3 supplied by Fluka Chemical Co (lot. No. not stated in report)	>97%		A6.6.1/03 (genotoxicity/mutagenicity studies)
1α,25-Dihydroxy vitamin D3 (Calcitriol) Rocaltrol®, Roche	Not stated		A6.8.1/02, A6.8.1/03, A6.8.2/2 (Studies on reproductive effects)
Vitamin D3 tablets as follows: multivitamin	400 IU, 1600 IU, 3600 IU		A6.8.1/04 (clinical trial during pregnancy) ;

tablets containing 400 IU of Vitamin D3 per tablet and an additional Vitamin D3 tablets containing 0 IU (placebo), 1600 IU or 3600 IU			Hollis B.W, Johnson D., Hulsey T.C., Ebeling M. and Wagner C.L. (2011) GMP manufactured. Vitamin D3 concentration in the tablets was verified by the company every 6 months and by an independent laboratory chosen by the investigators
Vigantol Oil, Merk KGaA, Germany	20697 IU/mL 96.5% of labelled concentration		A6.8.1/05 (TK study in pregnant women)
Vitamin D3	10000 IU	No details on purity and supplier.	A6.2-3
C9756	Purity and batch was not stated	No details on purity, Sigma Chemical Co., St Louis, MO, USA Catalog No. C9756	A6.5/02 (26 week study in rats)
24(R),25-(OH)2D3	Purity and batch was not stated	No details on purity , Supplied by Kureha Chemical Co. Ltd, Tokyo, Japan	A6.5/03

2. PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	603-180-00-4	Colecalciferol, Vitamin D3	200-673-2	67-97-0	Acute Tox. 3* Acute Tox. 3* Acute Tox. 2* STOT RE 1	H301 H311 H330 H372**	GHS06 GHS08 Dgr	H301 H311 H330 H372**			
Dossier submitters proposal	603-180-00-4	Colecalciferol, Vitamin D3	200-673-2	67-97-0	Change: Acute Tox. 2 (all routes) STOT RE 1 Add: Carc. 2 Muta. 2	H300, H310, H330 H372 Add: H351 H341	GHS06 GHS08 Dgr	H300, H310, H330 H372 H351 H341		STOT RE 1; H372: C ≥ 0,6 % STOT RE 2; H373: 0,06 % < C < 0,6 %	

Table 7: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	hazard class not assessed in this dossier	No
Oxidising gases	hazard class not assessed in this dossier	No
Gases under pressure	hazard class not assessed in this dossier	No
Flammable liquids	hazard class not assessed in this dossier	No
Flammable solids	hazard class not assessed in this dossier	No
Self-reactive substances	hazard class not assessed in this dossier	No
Pyrophoric liquids	hazard class not assessed in this dossier	No
Pyrophoric solids	hazard class not assessed in this dossier	No
Self-heating substances	hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	hazard class not assessed in this dossier	No
Oxidising liquids	hazard class not assessed in this dossier	No
Oxidising solids	hazard class not assessed in this dossier	No
Organic peroxides	hazard class not assessed in this dossier	No
Corrosive to metals	hazard class not assessed in this dossier	No
Acute toxicity via oral route		Yes
Acute toxicity via dermal route		Yes
Acute toxicity via inhalation route		Yes
Skin corrosion/ irritation	hazard class not assessed in this dossier	No
Serious eye damage/eye irritation	hazard class not assessed in this dossier	No
Respiratory sensitisation	hazard class not assessed in this dossier	No
Skin sensitisation	hazard class not assessed in this dossier	No
Germ cell mutagenicity		Yes
Carcinogenicity		Yes
Reproductive toxicity	data not conclusive	Yes
Specific target organ toxicity-single exposure		Yes
Specific target organ toxicity-repeated exposure		Yes

Aspiration hazard	hazard class not assessed in this dossier	No
Hazardous to the aquatic environment	hazard class not assessed in this dossier	No
Hazardous to the ozone layer	hazard class not assessed in this dossier	No

3. HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Classification of the active substance for reproductive effects has been considered previously. The study considered by the Commission Working Group on Classification and Labelling of Dangerous Substances and by the Specialised Experts is the same calcitriol study in rats and rabbits that is reported also in this dossier. The meeting decided that cholecalciferol should not be classified for developmental toxicity or fertility effects. The basis for this decision is summarised in the document denoted ECBI/76/00 - Rev. 3, 30.08.2001; ECBI/59/00 – Add. 1 - Rev. 2, 11.04.2001.

Regarding effects on development, this document informs: "*The Specialised Experts discussed ergocalciferol and colecalciferol in parallel. The Specialised Experts noted that there were few studies that had been adequately designed, conducted and reported that could be considered for purposes of classification of effects on the development. A majority of the Specialised Experts recommended no classification of cholecalciferol for effects upon the development. The arguments were that there is no evidence suggesting that the substance is teratogenic in humans, even at high doses. In animal studies, effects on development but not malformations occurred at dose levels, for which hypercalcemia had been demonstrated or must have been present. Other lesions in fetuses were considered non-specific and secondary to maternal toxicity. A large minority of Experts in favour of Category 3 argued that external, visceral and skeletal abnormalities observed in rabbit fetuses after administration of high doses of the active metabolite 1α,25-dihydroxyvitamin D3 (calcitriol) are specific (McClain et al., 1980) and should lead to classification"* (citation from ECBI/ECBI/59/00 - Rev. 2).

With respect to fertility effects, the document informs: "*The Specialised Experts discussed ergocalciferol and colecalciferol in parallel. The Specialised Experts noted that there were few studies that had been adequately designed, conducted and reported that could be considered for purposes of classification. The Specialised Experts unanimously agreed that classification of ergocalciferol and colecalciferol for effects upon fertility is not warranted, as no valid and conclusive evidence is available from investigations on humans and animals."* (citation from ECBI/ECBI/59/00 - Rev. 2).

Cholecalciferol has existing classifications under DSD which were translated into CLP classifications when CLP entered into force. No specific concentration limits exist. The existing classification is (ATP/CLP00):

Acute Tox. 3*; H301 and H311

Acute Tox. 2*, H330

STOT RE 1, H372**

4. JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

The substance is an active substance in the meaning of Regulation (EU) No 528/2012 repealing Directive 98/8/EC and a justification is thus not required according to Article 36 of Regulation (EC) No 528/2012 (CLP).

5. IDENTIFIED USES

The substance is intended to be used in the context of Regulation (EC) No 528/2012 as a rodenticide Product Type (PT 14). Vitamin D3 is currently being evaluated as new biocidal active substance under Directive 98/8/EC for use as rodenticide (PT 14).

6. DATA SOURCES

The data sources used for this report include the dossier compiled by the applicant who supported the review under Regulation (EU) No 528/2012 repealing Directive 98/8/EC 98/8/EC. This dossier is mainly based on open literature data and proprietary data. Data from the dossier are discussed in the following sections but the study summaries (denoted Doc IIIA) of the draft Competent Authority Report (CAR) for biocides are included in a confidential attachment to the technical dossier (IUCLID) since the report has not yet been subjected to the peer-review process under Regulation (EU) No 528/2012. Whenever possible, references are given both to Doc IIIA of the draft CAR and to published information. Additional published information has been added by the dossier submitter whenever available and considered relevant.

7. PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties

No table included since physical hazards is not assessed in this dossier.

8. EVALUATION OF PHYSICAL HAZARDS

These hazard classes are not assessed in this dossier

9. TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 9: Summary table of toxicokinetic studies

Method	Results	Remarks	Reference
No ADME study available. A summary of the information provided by the biocides applicant is presented below.			IIIA6.2 Safe Upper Limits for Vitamins and Minerals (2003) Vitamins in Medicine Volume 1

There is no guideline-compliant study of metabolism in animals available, but information on Vitamin D3 (cholecalciferol) pharmacokinetics and metabolism can be found in the peer reviewed literature.

Cholecalciferol is used extensively in therapeutics, to correct Vitamin D3 deficiency. It is an essential vitamin, it is synthesised in the skin in sunlight and it is thus naturally present in the body. Based on information from publications available in the open literature the toxicokinetic profile of cholecalciferol can be summarised as:

Absorption and distribution

Dietary vitamin D3 is absorbed through the small intestine and then transported in the lymph. In the plasma, vitamin D3 (from either diet or the skin) is bound to a protein synthesised in the liver, Vitamin D-binding Protein (DBP), for transport to the liver. The form of vitamin D3 reaching the liver is 25-hydroxylated. In plasma 25-hydroxycholecalciferol circulates bound to DBP (α2-globulin).

The estimated T_{max} following a single oral dose (70,000 IU) of cholecalciferol to pregnant and non-pregnant women is 11 days, based on 25-hydroxycholecalciferol (see table 10.10.f). Approximately 30% of the AUC occurred post-28 days.

Metabolism and excretion

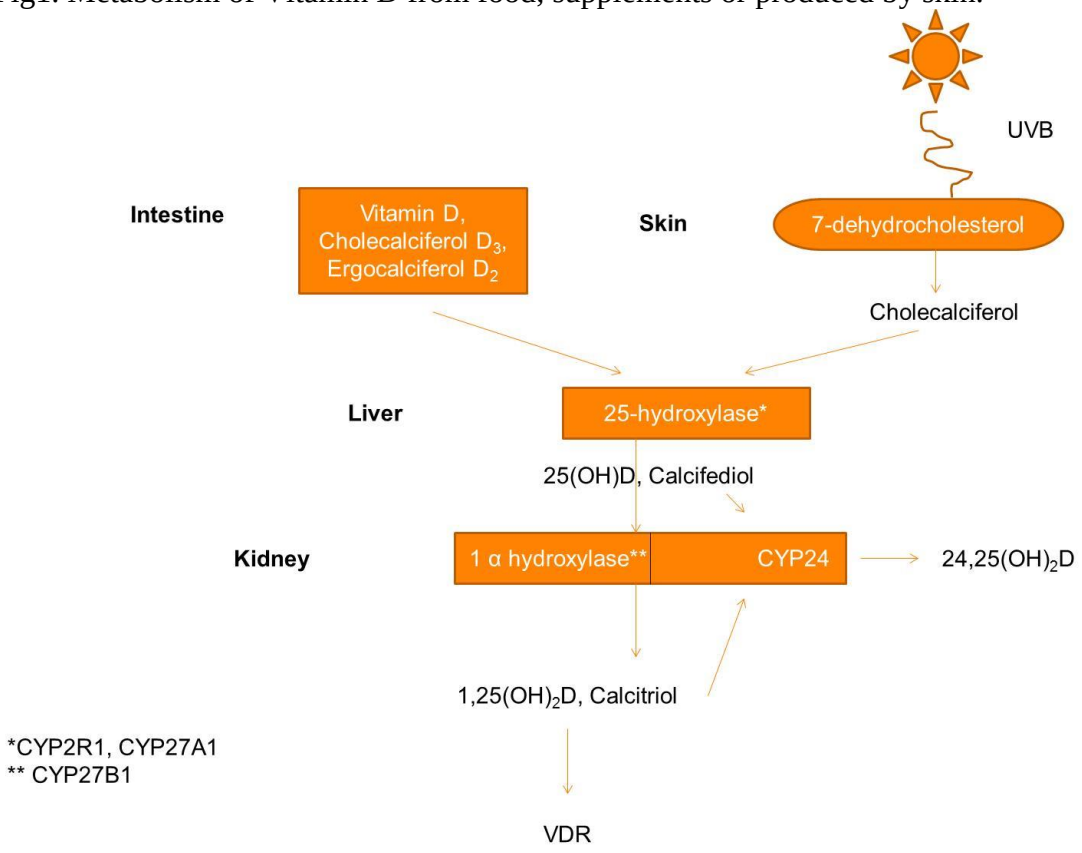
As stated above, cholecalciferol is hydroxylated in the liver at the 25-carbon atom by a cholecalciferol-25-hydroxylase enzyme. The product of this hydroxylation, 25-hydroxycholecalciferol, is also known as calcidiol or calcifediol.

The 25-hydroxycholecalciferol is transported from the liver to the kidney, where it undergoes a second hydroxylation by 25-OH cholecalciferol–1-hydroxylase (1-OH-ase) and produces 1α, 25-Dihydroxycholecalciferol (calcitriol). This metabolite of cholecalciferol regulates intracellular and extracellular concentrations of calcium in order to maintain a physiological range. This is accomplished by stimulation of intestinal calcium and phosphate transport, by mobilisation of phosphorous and calcium from bone and by other functions commonly attributed to cholecalciferol (EFSA scientific opinion, 2012). The rate of conversion to 1α, 25-Dihydroxycholecalciferol by the kidney is dependent on parathyroid hormone (PTH) which is secreted in response to low plasma calcium levels.

1α-hydroxylation of 25-hydroxycholecalciferol has been demonstrated in *in vitro* cell culture systems including neonatal keratinocytes, chick embryonic calvaria, human bone cells, human osteosarcoma cells and macrophages. Intracellular VDR receptors with ligand selectivity for calcitriol and related metabolites are found in nearly all vertebrates, including mammals (human, mouse). Enzymes that metabolise vitamin D and VDR receptors have been identified in human male reproductive tissue.

Kidney mitochondria contain another enzyme, 25-OH-cholecalciferol–24-hydroxylase (24-OH-ase) which hydroxylates 25-hydroxycholecalciferol to form 24, 25-dihydroxycholecalciferol. Less is known on the function of this metabolite. It has been suggested that the 24-hydroxylation is the initial step in the degradation process. However, the final degradation product of cholecalciferol is calcitroic acid, which is excreted in urine.

Fig1. Metabolism of Vitamin D from food, supplements or produced by skin.



9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Cholecalciferol can be absorbed orally and via skin. The vitamin D receptor (VDR) and vitamin D-metabolising enzymes are widespread in the tissues of the human body and among vertebrates. Therefore, hazard responses resulting from excessive exposure to cholecalciferol are expected to be similar in both humans and vertebrates and in widespread tissues.

The data used to support the classification proposals made in this report include studies with cholecalciferol as well as studies performed with certain metabolites of cholecalciferol (see table below). Calcitriol is currently considered to be the hormonally active metabolite binding to the VDR receptor and studies performed with this metabolite are therefore considered relevant for estimating the hazard of cholecalciferol (this is further discussed in the section on reproductive toxicity).

Systematic steroid name[a]	Trivial name	Abbreviation in Fig 1	Used in toxicological study
(5*Z*,7*E*)-(3*S*)-9,10-seco-5,7,10(19)-cholestatrien-3-ol	cholecalciferol or vitamin D3	cholecalciferol or vitamin D3	Section 10.1 (all) Section 10.8 (all) Section 10.9 (90 day rat, 26 week rat, EFSA scientific opinion, systematic literature review) Section 10.10 (EFSA scientific opinion, systematic literature review, epidemiological data)
(5*Z*,7*E*)-(3*S*)-9,10-seco-5,7,10(19)-cholestatriene-3,25-diol	Calcidiol calcifediol 25-hydroxycholecalciferol	25(OH)D	-
(5*Z*,7*E*)-(1*S*,3*R*)-9,10-seco-5,7,10(19)-cholestatriene-1,3,25-triol	Calcitriol 1α,25-Dihydroxycholecalciferol 1,25-dihydroxy vitamin D3	1,25$(OH)_2$D	Section 10.10.1 fertility studies in rat, rabbits human data in scientific opinion by EFSA
n.a.[b]	24, 25-dihydroxycholecalciferol 24,25-dihydroxy vitamin D3 (24R)-Hydroxycalcidiol	24, 25 $(OH)_2$D	Section 10.9 57 week study in rat
	ergocalciferol (vitamin D2)	ergocalciferol D2	Section 10.10.2 published studies on developmental toxicity

[a]IUPAC Commission on the Nomenclature of Organic Chemistry (CNOC) and IUPAC-IUB Commission on Biochemical Nomenclature (CBN). The Nomenclature of Steroids, Revised tentative rules, 1967, Arch. Biochem. Biophys. 136, 13-35 (1970) amended 147, 4-7 (1971), Biochem. J. 113, 5-28 (1969) amended 127, 613-616 (1972), Biochemistry, 8, 2227-2242 (1969) amended 10, 4994-4995 (1971), Biochim. Biophys. Acta, 164, 453-486 (1968) amended 248, 387-390 (1971), Eur. J. Biochem. 10, 1-19 (1969) amended 25, 1-3 (1972) and - amendments incorporated - Pure Appl. Chem. 31, 285-322 (1972); also on pages 133-153 in [ref 3]. [Now available as a 1989 third edition.]

[b]Systematic name not available; IUPAC-name: (6R)-6-[(1R,3aS,4E,7aR)-4-[(2Z)-2-[(5S)-5-hydroxy-2-methylenecyclohexylidene]ethylidene]-7a-methyl-2,3,3a,5,6,7-hexahydro-1H-inden-1-yl]-2-methylheptane-2,3-diol

10. EVALUATION OF HEALTH HAZARDS

Cholecalciferol (vitamin D3) is one of the two main forms of vitamin D, the other main form is ergocalciferol (vitamin D2). The two forms only differ by their side chains on the sterol skeleton. Cholecalciferol is continuously produced in the skin with help from sunlight and is therefore naturally present in our bodies. Vitamin D is also supplied by intake of vitamin D-containing food. The endogenous production of vitamin D in humans is low during the winter season within the EU area and therefore extra sources of vitamin D, e.g. fortified food and other vitamin D supplements, may be needed for some individuals to avoid problems such as rickets. It is well known that vitamin D is important for calcium homeostasis and skeletal bone ossification processes but it also has other important physiological functions such as being involved in renin production and insulin production (discussed in EFSA scientific opinion, 2012). Vitamin D is thus essential for humans but endogenous levels differ between individuals due to differences in age, exposure to sunlight and dietary intake. Nevertheless, EFSA has concluded that intake of vitamin D from all sources are below the tolerable upper intake levels set at 100 µg/day in adults and 50 and 25 µg/day in children and infants respectively[1], levels below which only beneficial effects of vitamin D are expected.

In the following sections, effects resulting from exposure to cholecalciferol outside of the physiological range are discussed in order to assess the intrinsic hazards of the substance. When information refers to results from clinical trials, doses are often given in international units (IU) where 1 IU corresponds to 0.025 µg.

10.1 Acute toxicity - oral route

Table 10.1: Summary table of animal studies on acute oral toxicity

Route	Method Guideline	Species Strain Sex no/dose	Dose levels duration of exposure	LD_{50} value	Remarks: target organs, acute toxicity effects, classification etc.	Reliability factor	Reference
Oral	Similar to OECD guideline 401	Rat; Sprague-Dawley; males & females; 6/sex/group	146, 219, 329, 493, 739, 1109 mg/kg bw Up to 38 days	LD_{50} Males: 352 mg/kg	Doses confirmed by analysis ***146 mg/kg bw*** (Diarrhoea, lacrimation) ***219 mg/kg*** (Diarrhoea, hypoactivity, emaciation, oily yellow stained anal region, red stained nose and mouth, **death** 1/6) ***328.5 mg/kg*** (Diarrhoea, hypoactivity, ataxia,	1	IIIA 6.1.1/01

[1] Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. EFSA Journal 2012;10(7):2813 [45 pp.].doi:10.2903/j.efsa.2012.2813

Route	Method Guideline	Species Strain Sex no/dose	Dose levels duration of exposure	LD_{50} value	Remarks: target organs, acute toxicity effects, classification etc.	Reliability factor	Reference
					emaciation, oily yellow stained anal region, red stained nose, end of tail missing, **death** 3/6) ***492.6 mg/kg*** (Diarrhoea, hypoactivity, emaciation, oily yellow stained anal region, red stained nose and mouth, lacrimation, **death** 5/6)		
				Females: 619 mg/kg	Doses confirmed by reanalysis ***146 mg/kg bw*** (hypoactivity, emaciation, ataxia) ***219 mg/kg*** (hypoactivity, emaciation, ataxia, lacrimation, red staining around eyes) ***328.5 mg/kg*** (Diarrhoea, hypoactivity, emaciation, ataxia, lacrimation, red staining around eyes, brown stained anal region, yellow stained abdomen) ***492.6 mg/kg*** (Hypoactivity, emaciation, ataxia, lacrimation, red stained mouth and nose, yellow stained anal region, bradypnoea, **death 1/6**		

Route	Method Guideline	Species Strain Sex no/dose	Dose levels duration of exposure	LD_{50} value	Remarks: target organs, acute toxicity effects, classification etc.	Reliability factor	Reference
					738.9 mg/kg (Diarrhoea, hypoactivity, emaciation, ataxia, lacrimation, red stained mouth and nose, brown stained anal region, dyspnoea, piloerection, tremors, **death 5/6**) ***1108.7 mg/kg*** (Diarrhoea, hypoactivity, emaciation, ataxia, lacrimation, brown stained anal region, dyspnoea, **death 6/6**) Pathology: Necropsy findings of decedent animals included mottled dark red/red or tan lungs, kidneys, heart. In animals examined at study termination light gray foci often raised and firm, were noted in lungs, kidneys and heart; linear tan areas in hearth and stomach, and mottled lungs were also noted.		
Oral The study is largely considered reliable for the purpose of assessing acute oral toxicity despite lack of guideline claim and GLP since the study follows the concurrent guideline and results were clearly reported.	Similar to OECD guideline 401	Wistar rats Male/female 5/sex/group	0, 12.5, 25, 50, 100, 200 mg/kg 14 days	Males: 35 mg/kg bw (95% confidence limits: 24 – 53 mg/kg bw) Females: 47 mg/kg bw (95% confidence limits: 28 – 79 mg/kg bw)	Purity of test substance unknown **<u>Males and females:</u>** ***0 mg/kg bw*** (no clinical signs) ***12.5 mg/kg bw*** (Dispersion in cage, apathy, decreased alertness and startle response, decreased locomor activity, abnormal body posture and gait,	2-3	IIIA 6.1.1/02

Route	Method Guideline	Species Strain Sex no/dose	Dose levels duration of exposure	LD_{50} value	Remarks: target organs, acute toxicity effects, classification etc.	Reliability factor	Reference
The reliability is lowered by the fact that the purity of the test substance is unknown.					piloerection, passiveness, decreased body tone, ptosis, paralysis, loss of grooming, respiration difficulties, degree mostly slight, reversible day 4-5) ***25 mg/kg bw/day*** (as above, degree moderate, **deaths** 1/5 males and 1/5 females) ***50 mg/kg bw*** (as above, degree moderate, **deaths** 4/5 males and 4/5 females) ***100 mg/kg bw*** (as above, degree moderate, **deaths** 5/5 males and 5/5 females) ***200 mg/kg bw*** (as above incl. decreased pinna reflex and lacrimation, degree moderate to severe, **deaths** 5/5 males and 3/5 females) Pathology: Rats that died during, or were killed after, the observation period had at autopsy at 25 mg/kg and above calcification (white spots) of heart, spleen, kidney and blood vessels.		

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

The purities of the test substances used in the acute oral toxicity studies were not given. However, in the study using Sprague Dawley rats, the doses were confirmed by vitamin D3 activity analysis giving some indication of the purity (although there were no details of the analysis method included in the study report). The result of this study falls within category 3 for acute toxicity if the LD_{50} is based on the lower end of the 95% confidence limits in males (i.e. 268-484 mg/kg bw). The study thus confirms the existing classification in category 3. However, the LD_{50} values set in the study with Wistar rats were lower and it is not possible to conclude if the higher toxicity in this study was due to a higher purity of the test substance or if it was related to the type of strain used. The study report describing the study in Wistar rats does not include a vitamin D3 activity analysis but pathology confirmed hypercalcinosis at the lethal dose which at least supports that the substance tested indeed was cholecalciferol. The LD_{50} set in Wistar rats is 35 (with 95% confidence limits of 24 – 53 mg/kg bw) in males and 47 mg/kg bw (with 95% confidence limits of 28 – 79 mg/kg bw) in females. A third study on acute oral toxicity submitted was not considered acceptable for addressing the endpoint due to the use of very low doses (study IIIA 6.1.1-03 in the confidential attachment). Additional information on LD_{50} values can be found in rodenticide efficacy tests (in Doc IIIA, section 5 of the confidential annex I). The LD_{50} values set in these studies are in the same range as the LD_{50} values set in Wistar rats.

10.1.2 Comparison with the CLP criteria

Study using Sprague-Dawley rats:

The LD_{50} of 352 mg/kg bw in males falls within the range for category 4 (i.e. 300 <ATE≤ 2000 mg/kg bw). The 95% confidence limits are 268-484 mg/kg bw thus the lower limit falls just within the range for category 3 (50 < ATE ≤ 300 mg/kg bw). The female LD_{50} of 629 mg/kg bw (with 95% confidence limits of 495-782 mg/kg bw) also falls within the range for category 4. The doses were confirmed by analysis of the test substances but the purity was not given.

Study using Wistar rats:

The LD_{50} of 35 mg/kg bw (with 95% confidence limits of 24 – 53 mg/kg bw) in males and 47 mg/kg bw (with 95% confidence limits of 28 – 79 mg/kg bw) in females fall within the range for category 2 (i.e. 5 < ATE ≤ 50 mg/kg bw). The purity of the test substance is unknown and the reliability of the study is thus considered to be lower than the other study. However, the LD_{50} values set are supported by the results from rodenticide efficacy tests.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

The existing classification of cholecalciferol as Acute Tox. 3* in Annex VI of CLP is a minimum classification (CLP Regulation section 1.2.1). Taking into consideration the acute toxicity data presented here, classification in acute toxicity category 2, H300 (fatal if swallowed) is proposed based on the LD_{50} of 35 mg/kg bw set in the study using Wistar rats.

10.2 Acute toxicity - dermal route

Table 10.2 Summary table of animal studies on acute dermal toxicity

Route	Method Guideline	Species Strain Sex no/dose	Dose levels duration of exposure	LD_{50} value	Remarks: target organs, acute toxicity effects, classification etc.	Reliability factor	Reference
Dermal	Similar to OECD guideline 402	Wistar rats Male/female 5/sex/group	37.5, 75, 150, 300, 600 mg/kg 35 days	LD50: male: 61 mg/kg female: 185 mg/kg.	Purity of test substance unknown **Males:** ***0 mg/kg bw*** (Decreased locomotor activity, abnormal body posture and gait and diminished grooming. Disappeared 3 days after dosing.) ***37.5 mg/kg bw*** (**deaths** 1/5, Dispersion in cage, apathy, respiration difficulties, decreased alertness and startle response, decreased locomor activity, abnormal body posture and gait, piloerection, decreased pinna-reflex, passiveness, decreased body tone, ptosis, paralysis, diminished grooming, death. Diminished hair-growth on treated area. Degree mostly slight). ***75 mg/kg bw*** (**deaths** 5/5, clinical signs same as above) ***150 mg/kg bw*** (**deaths** 3/5, clinical signs same as above) 300 mg/kg bw (**deaths** 5/5, clinical signs same as above) **Females:** ***0 mg/kg bw*** (Decreased locomotor activity, abnormal body posture and gait and diminished grooming. Disappeared 3 days after dosing.) ***75 mg/kg bw*** (**deaths** 1/5, Dispersion in cage, apathy, respiration difficulties, decreased alertness and startle response, decreased locomotor activity, abnormal body posture and gait, piloerection, decreased pinna-reflex, passiveness,	2-3	IIIA 6.1.2/01

Route	Method Guideline	Species Strain Sex no/dose	Dose levels duration of exposure	LD_{50} value	Remarks: target organs, acute toxicity effects, classification etc.	Reliability factor	Reference
					decreased body tone, ptosis, paralysis, diminished grooming, death. Diminished hair-growth on treated area. Degree mostly slight). ***150 mg/kg bw*** (**deaths** 2/5, clinical signs same as above) 300 mg/kg bw (**deaths** 4/5, clinical signs same as above) ***600 mg/kg bw*** (**deaths** 4/5, clinical signs same as above)		

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

The acute dermal LD_{50} values calculated were 61 mg/kg bw (with 95% confidence limits of 39 – 95 mg/kg bw) in male rats and 185 mg/kg bw (with 95% confidence limits of 103 – 332 mg/kg bw) in female rats. The purity was not known. The rats were individually housed during exposure and the test site was occluded to prevent contribution to toxicity by ingestion of cholecalciferol. Clinical signs were mainly indicative of decreased motor activity and motor coordination, respiration difficulties and muscle weakness. Weight loss was reported and also heart calcification (hard with white spots) in the deceased or euthanized animals. The dermal study in rats thus indicates that cholecalciferol has an inherent property of being toxic also via this route and casues similar effects as those observed after oral and inhalation exposure including characteristic white spots in heart. There are no dermal absorption studies in rats and it is therefore not known if rats have much higher dermal absorption of concentrated active substance.

10.2.2 Comparison with the CLP criteria

The LD_{50} values in Wistar rats, i.e. 61 mg/kg bw in males (with 95% confidence limits of 39 – 95 mg/kg bw) and 185 mg/kg bw (95% confidence limits: 103 – 332 mg/kg bw) in females fall within the range for category 2 ($50 < ATE \leq 200$ mg/kg bw). The lower 95% confidence interval limit for males falls within category 1 (i.e. $ATE \leq 50$ mg/kg bw) but as the purity of the test substance is unknown, the exact LD 50 values cannot be determined.

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

The existing classification of cholecalciferol as Acute Tox. 3* (H311) in Annex VI of CLP is a minimum classification (CLP Regulation section 1.2.1). The criteria for classification in category 1 could be considered fulfilled if the lower confidence limit for the LD_{50} set in male rats is used as the

ATE. However, the LD_{50} values set for both male and female rats fall within the range for category 2. Moreover, effects were similar to those observed in the acute oral and inhalation toxicity studies and dermal toxicity rarely exceeds oral toxicity and toxicity via inhalation. Therefore, the acute dermal toxicity of cholecalciferol is proposed to fulfil criteria for classification in category 2.

10.3 Acute toxicity - inhalation route

Table10.3 Summary table of animal studies on acute inhalation toxicity

Route	**Method** **Guideline**	**Species** **Strain** **Sex** **no/dose**	**Dose levels** **duration of exposure**	**LD_{50} value**	**Remarks: target organs, acute toxicity effects, classification etc.**	**Reliability factor**	**Reference**
Inhalation	OECD guideline 403	Wistar rats Male/female 5/sex/group	136.7 - 380 mg/m³ 4 hours exposure 35 days , nose-only	LC_{50} 0.13 – 0.38 mg/L	Purity 98.6% ***0.14 mg/l*** (deaths 2/5 males, 1/5 females, , increased respiratory rate, respiratory difficulties, temporarily increased locomotor activity up to 24 h after treatment where after the locomotor activity was suppressed, apathy, humped back, decreased body tone (both sexes), decreased limb tone (females), loss of grooming, increased touch response, piloerection, decreased feed consumption, decreased urination and defaecation frequencies. Signs were slight to moderate in intensity and diminished over time, were absent day 20-32 in surviving animals.) ***0.15 mg/l*** (deaths 4/5 males, 1/5 females, same clinical signs as above, moderate in intensity absent in survivors day 16 to 20) ***0.2 mg/l*** (deaths 2/5 males, 3/5 females, clinical signs not described) ***0.4 mg/l*** (deaths 5/5 males, 3/5 females, same clinical signs as above, in addition ptosis and staggering gait, slight to moderate intensity, diminishing over time and were absent day 21)	1	**IIIA 6.1.3**

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

Under the conditions of the acute inhalation study available, the LC_{50} after a 4-hour exposure and a 35-day observation period was estimated to be in the range of 130 – 380 mg/m^3 (0.13-0.4 mg/L). Post-exposure observations of clinical symptoms showed systemic effects indicative of effects on the mood, motor activity and coordination, posture, muscle tone and the autonomic nervous system. However, since mortality occurred in some of the animals at the same dose levels, these symptoms are considered to reflect general toxicity rather than a specific effect on the nervous system. Pathology findings considered to be treatment related include:

- male rats: pale kidneys with roughened surface, white spots and areas in the myocardium, white area in the stomach and red spots on the lungs.

- female rats: pale kidneys with roughened surface, white spots and areas in the myocardium, white area in the stomach and red spots on the lungs.

No dose related changes of the lung weight of the males were observed. Among females there was an increase of both absolute and relative lung weights. The macro- and microscopic observations revealed calcification of the visceral organs and the lungs at all exposure levels. There were no signs of respiratory irritation. Body weights of surviving animals were reduced in exposed groups up to 3 weeks after treatment. Thereafter the body weights of both male and female rats resumed to control values. Mortality occurred at all exposure levels, between 48 hours and 7 days after exposure. The mortality pattern in males did not show a clear dose-response but indicated a slightly higher sensitivity of males compared to females.

10.3.2 Comparison with the CLP criteria

The LC_{50} is within the range of 0.13-0.15 mg/l for males and 0.14 to 0.4 mg/l for females. These values fall within the range for classification in category 2, i.e. 0.05 < ATE ≤ 0.5 mg/l (dust and mist).

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

The existing classification of cholecalciferol as Acute Tox. 2* (H330) in Annex VI of CLP is a minimum classification (CLP Regulation section 1.2.1). Based on the results of the study in Wistar rats, classification as Acute Tox. 2, H330: fatal if inhaled is proposed.

10.4 Skin corrosion/irritation

Hazard class not assessed in this dossier

10.5 Serious eye damage/eye irritation

Hazard class not assessed in this dossier

10.6 Respiratory sensitisation

Hazard class not assessed in this dossier

10.7 Skin sensitisation

Hazard class not assessed in this dossier

10.8 Germ cell mutagenicity

Table 10.8.a: Summary table of mutagenicity/genotoxicity tests *in vitro*

Method, guideline, deviations if any	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
OECD 471	Not. Stated But purity stated as: ≥ 97%	Reliability:2-3 *Saccharomyces cerevisiae* Strain D4 5.0, 0.5, 0.05, 0.005, 0.0005% w/v	Negative	IIIA 6.6.1/02
OECD 471	Not. stated But purity stated as: ≥ 97%	Reliability:2-3 *Salmonella typhimurium* TA98, 100, 1535, 1537, 1538 5.0, 0.5, 0.05, 0.005, 0.0005% w/v	Negative	IIIA 6.6.1/02

OECD 471	D0074	Reliability:1 *S. typhimurium* TA98, 100, 1535,1537 *E.coli* WP2 uvrA 78 – 5000 µg/plate	Positive Mutagenic activity was noted in two of the bacterium tester strains (S. typhimurium TA100 with S9 mix at >1250 µg/plate, and TA1535 with S9 mix at >2500 µg/plate (x2 response and x3 response respectively) The criteria for a positive response given in the report were reproducible dose dependence or a >2x response for TA100 and >3x response for TA 1535 Reproducible dose dependent mutagenicity was observed in TA100 (and 1535, initial –S9 and +S9 confirmatory test). Precipitate / slight precipitate was observed in Initial Mutation Test and Complementary Mutation Test in all examined bacterial strains at 5000 and 3750 µg/plate with/without metabolic activation and in Confirmatory Mutation Test in all examined bacterial strains at 5000 µg/plate with metabolic activation. Slight inhibitory, cytotoxic effect of the test item (reduced / slightly reduced background lawn development) was observed in the Initial Mutation Test and Complementary Mutation Test in all examined bacterial strains at 5000 µg/plate with and without metabolic activation and in the Confirmatory Mutation Test in S. typhimurium TA98 at 5000 µg/plate without metabolic activation.	IIIA 6.6.1/01
No guideline but the standardised protocol adopted by the National Toxicology Program (NTP) was used.		Deficiencies in reporting (study accepted as supplementary data only) Reliability:3 *S. typhimurium* TA98, 100, 1535,1537 33-10000 µg/plate	Negative	IIIA 6.6.1/03 Mortelmans, K., *et al* (1983) environmental Mutagenesis, vol 8

OECD 473	D0074	5-400 µg/mL	Negative No induction of chromosome aberrations (Chinese hamster V79 cells) Cholecalciferol was tested up to cytotoxic concentrations. Cytotoxicity was observed at 300 and 250 µg/mL without metabolic activation (relative survival 18 and 37%, respectively) and at 400 µg/mL with metabolic activation (relative survival 41%).	IIIA 6.6.2
OECD 476	D0074	Reliability:1 Mouse Lymphoma L5178 TK ±cells 10 – 325 µg/mL Concentrations tested (range): 5-325 µg/mL, test concentrations were based on an initial cytotoxicity test and on test substance solubility.	Negative No biologically relevant or statistically significant increase in the mutation frequency was observed. No significant dose response to the treatment was indicated by the linear trend analysis Assay 1, 3-hour treatment with metabolic activation: 325; 300; 275; 250; 225; 200, 175; 150; 125; 100; 75 and 50 µg/mL Excessive toxicity> 225 µg/mL: relative survival value of 17% at 225µg/mL. Assay 1, 3-hour treatment without metabolic activation: 325; 300; 275; 250; 225; 200, 175; 150; 125; 100; 75 and 50 µg/mL. Relative survival value of 11% at 200µg/mL Assay 2, 3-hour treatment with metabolic activation: 325; 300; 275; 250; 225; 200, 175; 150; 125; 100; 75 and 50 µg/mL Relative survival value of 28% at 225µg/mL Assay 2, 24-hour treatment without metabolic activation: 80; 70; 65; 60; 55; 50; 45; 40; 35; 30; 25; 20; 10 and 5 µg/mL. Relative survival value of 7% at 35µg/mL.	IIIA 6.6.3

Table 10.8.b: Summary table of mutagenicity/genotoxicity results *in vitro*

Strain TA100 (study IIIA6.6.1/01).

Concentration [µg/plate]	Initial Mutation Test		Confirmatory Mutation Test	
	Mean number of revertants		Mean number of revertants	
	- S9	+ S9	- S9	+ S9
Untreated control	122.3	111.0	101.7	94.3
DMSO	118.3	116.3	94.0	106.3
Distilled water control	118.3	-	106.7	-
5000	211.3	**305.0**	99.3	**245.7**
3750	221.7	**299.7**	94.7	204.3
2500	182.7	**265.3**	98.7	173.3
1250	139.7	201.7	105.3	125.0
625	123.7	175.3	107.3	135.7
312.5	124.3	158.0	107.7	115.0
156.25	115.3	139.7	104.0	114.0
78.125	100.0	123.0	107.7	114.7
PC	1477.3	2330.7	1440.0	2214.7

PC (positive controls): without S9-mix SAZ; with S9-mix 2-AA

Strain TA1535 (study IIIA6.6.1/01)

Concentration [µg/plate]	Initial Mutation Test		Confirmatory Mutation Test		Complementary Mutation Test
	Mean number of revertants		Mean number of revertants		Mean number of revertants
	- S9	+ S9	- S9	+ S9	- S9
Untreated control	11.0	15.3	12.0	9.0	9.0
DMSO	6.0	12.0	9.7	11.0	8.3
Distilled water control	8.3	-	9.3	-	8.7
5000	**19.3**	14.7	5.0	28.0	**40.3**
3750	**18.7**	22.0	7.0	27.3	**29.3**
2500	**18.7**	16.0	8.3	19.3	12.0
1250	12.7	15.0	8.7	12.7	12.3
625	7.7	10.3	10.7	17.0	9.0
312.5	10.3	11.7	12.3	11.7	7.7
156.25	13.0	12.0	8.7	10.0	8.7
78.125	8.3	11.3	8.7	13.7	7.7
PC	1354.7	205.3	1206.7	204.7	1280.7

PC (positive controls): without S9-mix SAZ; with S9-mix 2-AA

Table 10.8.c: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells *in vivo*

Method, guideline, deviations if any	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Combined bone marrow micronucleus test (OECD 474) and Comet assay	D0074	Reliability 1	Bone marrow micronucleus test: negative Comet assay: positive. Increased DNA migration in liver cells was observed in the 7.5 mg/kg bw and 15 mg/kg dose. No increased migration in duodenum. See table 10.8.d below for detailed results.	IIIA6.6.4/6.6.5/ 6.6.6

Table 10.8.d: Summary table of mutagenicity/genotoxicity test results in mammalian somatic *in vivo*

Type of test Method	Results	Remarks	Reference
Combined bone marrow micronucleus test (OECD 474) and Comet assay Reliability 1 Rat Wistar 3/sex/group (range-finder study) 6 M/group (main study) 3 applications (dosed at Day 1,2,3) 0, 3.75, 7.5, 15 mg/kg bw Sampling times: Day 3 (3 hrs after last dose)	Bone marrow micronucleus test: negative Comet assay: positive. Increased DNA migration in liver cells was observed in the 7.5 mg/kg bw and 15 mg/kg dose. No increased migration in duodenum. Comet analysis of liver resulted in increases in % tail intensity and tail moment following dosing at 7.5 and 15 mg/kg/day. The increases in % tail intensity were statistically significant when compared to the concurrent vehicle control group and also demonstrated a significant linear trend, although the degree of DNA damage did not increase when the dose level was increased from 7.5 to 15 mg/kg/day. The mean %tail intensity observed in groups dosed at 7.5 and 15 mg/kg/day (9.65 and 9.18, respectively) was outside the historical control range for the vehicle and laboratory in question. MTD 15 mg/kg bw based on mortality at higher doses. <u>3.75 mg/kg bw:</u> Calcium ↑24% Phosphor ↑70% Urea 16% Glucose ↓18% <u>7.5 mg/kg bw:</u> ↓ Bw loss 3.6% Calcium ↑37 % Phosphor ↑70% Urea 126% Glucose ↓31% ↑AST 81% ↓ALT 27% No adverse histopathology effects in liver but ↓ Glycogen vacuolation Myositis in duodenum in 2/6 males (grade minimal to slight) <u>15 mg/kg bw:</u> ↓ Bw loss 7.8% ↑AST 119% Calcium ↑31% Phosphor ↑55% Urea 314% Glucose ↓23% ↓ALT 49% No adverse histopathology effects in liver but ↓ Glycogen vacuolation ↓ Inflammatory cell foci of liver Myositis in duodenum in 6/6 males (grade minimal to slight).	No cytotoxicity effect was seen in bone marrow. ↑ Alizarin red staining in one animal at 7.5 (mucosa of duodenum) and in in one animal at 7.5 and another at 15 mg/kg (muscularis of pylorus) There was no dose-related increase in % clouds or % cells with halos in the liver or duodenum following treatment with Cholecalciferol, thus demonstrating that Cholecalciferol did not cause excessive DNA damage (which can interfere with Comet analysis) in either the liver or duodenum following oral administration. A complete depletion of glycogen was noted in the liver of all animals of mid and high dose groups. Complete depletion of glycogen was also noted in the liver in one control animal, and the rest of the controls (5/6) showed *minimal* glycogen vacuolation. Thus, the effect could be a consequence of fasting.	IIIA6.6.4/ 6.6.5/ 6.6.6

Table 10.8.f Summary of Comet Assay Data – Duodenum

Group / Treatment (mg/kg/day)	Total No. Cells Scored	Tail Intensity	Tail Moment	Mean % Clouds	Mean % Cells with Halos
		Mean ± SEM	Mean ± SEM		
1M / Vehicle (0)	900	2.84 ± 1.42	0.30 ± 0.15	17.72	15.50
2M / Cholecalciferol (3.75)	900	1.43 ± 0.38 NS	0.16 ± 0.05	19.24	11.17
3M / Cholecalciferol (7.5)	850	2.75 ± 1.19 NS	0.32 ± 0.16	20.64	16.83
4M / Cholecalciferol (15)	900	1.39 ± 0.58 NS	0.16 ± 0.07	19.12	12.67
		A			
5M / Ethyl methanesulfonate (150)	900	10.52 ± 1.68**	1.40 ± 0.26	22.82	11.83
		S+			

NS: not significant; A: ANOVA, dose response and Dunnett's; **: $p \leq 0.01$; S+: Two-sample t-test

Table 10.8.g Summary of Comet Assay Data – Liver

Group / Treatment (mg/kg/day)	Total No. Cells Scored	Tail Intensity	Tail Moment	Mean % Clouds	Mean % Cells with Halos
		Mean ± SEM	Mean ± SEM		
1M / Vehicle (0)	750	5.10 ± 0.63	0.55 ± 0.07	11.31	8.80
2M / Cholecalciferol (3.75)	750	6.16 ± 0.41 NS	0.65 ± 0.06	11.42	8.00
3M / Cholecalciferol (7.5)	900	9.65 ± 0.70**	1.05 ± 0.08	12.57	8.83
4M / Cholecalciferol (15)	900	9.18 ± 1.40**	1.06 ± 0.15	13.19	11.17
		DR***, A			
5M / Ethyl methanesulfonate (150)	900	51.54 ± 3.76***	10.25 ± 1.08	21.27	13.00
		S+R			

NS: not significant; **: $p \leq 0.01$; DR: significant dose response test; ***: $p \leq 0.001$; A: ANOVA, dose response and Dunnett's; S+R: Two-sample t-test+ Rank-transformed data

Table 10.8.h: Summary table of human data relevant for germ cell mutagenicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Non guideline descriptive study (published paper)	Not relevant	Investigation of VDR receptors in human tissue samples (testes and sperm). mRNA detection (RT-PCR) and immunohistochemistry of VDR and vitamin D metabolizing enzymes (CYP2R1, CYP27A1, CYP27B1, CYP24A1) in testis, epididymis, seminal vesicle, prostate and spermatozoa	VDR and vitamin D metabolising enzymes were expressed in round and elongated spermatids, vesicles within caput epididymis, in glandular epithelium of cauda epidymis, seminal vesicle and prostate. VDR, CYP2R1, CYP27B1 and CYP 24A1 was expressed in mature spermatozoa.	IIIA.6.14 Vitamin D 3rd edition (2011)
Effects of Supplemental Vitamin D and Calcium on Oxidative DNA Damage Marker in Normal Colorectal Mucosa: A Randomised Clinical Trial Published study	Calcium (as calcium carbonate), 2g/day in 2 equal doses Vitamin D (VitD)3, 800 IU/day in 2 equal doses	Oral, 6 months exposure 92 patients (age 30-75 years) were included in the trial. The participants underwent a baseline rectal biopsy (of normal mucosa) and were randomly assigned to one of 4 treatment groups, with approximately 20-23 participants per group. 1. Placebo (free of Vitamin D, calcium, magnesium and chelating agents); 2. 800 IU/day VitD3; 3. 1000 mg/day calcium (as carbonate), 4. combined 800 IU VitD3 + 1000 mg calcium/day Blood was analysed by radioimmunoassay for 25-(OH) VitD3 and for 1,25(OH)2 VitD3. The biopsy samples were fixed, then immunostained for 8-OH-dG and for VDR.	Vitamin D3 supplementation for 6 months at 800 IU/day (20 µg/day) was associated with a decrease in 8 (OH) dG in biopsy tissue. The difference was statistically significant in a subset of patients with higher VDR expression. A slightly lesser decrease was seen in samples from patients receiving calcium but not vitamin D. There was an increase in 8-OH-dG labelling in the calcium plus vitamin D group relative to the placebo.	IIIA.6.6.4/02

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

The genotoxic potential of cholecalciferol was investigated in three standard *in vitro* test systems (Ames test, mammalian cell gene mutation test, mammalian chromosome aberration test) reinforced with an *in vivo* combined Comet and micronucleus assay. The *in vivo* combined Comet and micronucleus assay was submitted by the applicant during the biocide review of cholecalciferol and has been evaluated by the dossier submitter.

The potential for mutagenic activity was tested *in vitro* in three Ames tests. In the third study listed in table 10.8.a, the criteria for a positive response were fulfilled. The study was well-conducted and performed in accordance with ICI policy for Good Laboratory Practice (GLP) and OECD TG 471. The two other Ames test available were negative but both studies were considered as non-key studies of low reliability, one study because of deficiencies in reporting.

The potential for mutagenicity was also tested *in vitro* in a mammalian cell gene mutation test and in a mammalian chromosome aberration test. Negative responses were obtained in both tests. The studies were well-conducted and performed in accordance with GLP and guidelines.

Cholecalciferol was tested *in vivo* in a combined Comet and micronucleus assay. The study was performed in accordance with GLP and the draft OECD TG for Comet assay (December 2013). The result of the micronucleus assay was negative. However, since no cytotoxic effect was seen in the bone marrow it is not possible to conclude that the target tissue was exposed in the study although this seems likely considering that cells such as osteoblasts, osteoclasts and chondrocytes express VDR (Vitamine D Receptor). Nevertheless, the micronucleus assay is not considered to be the adequate *in vivo* follow up of a positive *in vitro* result in the Ames test since the micronucleus test detects structural and/or numerical damage to chromosomes rather than point mutations. In contrast, the result of the Comet assay was positive. Cholecalciferol induced an increase in DNA migration in the liver. The mean per cent tail intensity observed in groups administered 7.5 and 15 mg/kg/day (9.65 and 9.18, respectively) was outside of the historical control range for the vehicle and laboratory in question (the historical control range generated from 66 studies between July 2010 and August 2014 showed an upper limit of 5.51 and the historical control range generated from 6 studies between May 2008 and March 2013 showed an upper limit of 4.81). It could be argued that this positive result should be considered irrelevant and disregarded for classification since the doses where DNA migration occurred were above the maximum tolerable dose (MTD). The MTD was considered to be reached based on the following findings:

- Severe body weight losses occurred over a short period of 3 days in young rats which are supposed to grow rapidly at this stage of development.

-Severe alteration of several clinical chemistry parameters (i.e. increases in Ca/P, urea and liver enzymes) reflecting renal and muscular failure.

- Decreases in liver glycogen vacuolation considered to be hallmarks of cholecalciferol toxicosis, e.g. loss of appetite and the use of endogeneous glycogen.

Based on data from the dose-range finding study, 15 mg/kg/day was considered an appropriate estimate of the MTD since no deaths occurred at this level. However, one male administered 20 mg/kg bw animal was found dead prior to dosing on day 3 and the high dose chosen seems near a lethal dose considering that animals administered 10 mg/kg/day in a 28 day study died already at the first week of dosing (i.e. from day 3).

The applicant for the biocide review provided a position paper stating that the loss of glycogen in the livers of animals receiving 7.5 and 15 mg/kg bw/day was a consequence of pronounced weight loss indicating severe systemic toxicity (Bayer CropScience, 2014). However, the dossier submitter does not support this position since these effects, i.e. the observed body weight losses of 3.6% and 7.8 % at 7.5 and 15 mg/kg bw (compared to a body weight gain of 4.9% in controls) and effects on clinical chemistry parameters are not apparently linked to DNA damage. The changes in serum chemistry parameters stated to indicate a systemic toxicity included effects on calcium, phosphor, urea, glucose, AST and ALT levels. Calcium was increased between 24 and 37% at 3.75, 7.5 and 15 mg/kg bw respectively. Phosphor was increased by 70, 70 and 55% at 3.75, 7.5 and 15 mg/kg bw respectively. Urea was increased by 16-314% at 3.75, 7.5 and 15 mg/kg bw respectively. Glucose was decreased by 18, 31 and 23% at 3.75, 7.5 and 15 mg/kg bw respectively. The AST was increased (81% and 119% respectively) at these doses but the ALT was decreased (by 27% and 49% respectively). There is thus neither excessive effects nor a clear dose-correlation. The high increase in AST could instead be explained by the myositis observed around areas of necrosis in the duodenum.

Excessive toxicity may give false positive results due to induction of necrosis followed by hyperplasia. However, although effects occurred at doses above the MTD, there was no increased frequency of inflammation observed in the livers of animals treated with 7.5 mg/kg bw and 15 mg/kg (the frequency was in fact decreased) thus the increased DNA migration in the liver cannot be explained by an excessive toxicity. Liver toxicity was not reported in the 28 day study (but the study design was limited since it was a dose finding study) or in the acute oral toxicity studies. Complete glycogen depletion was noted in the liver at 7.5 and 15 mg/kg bw and the applicant considers this effect related to systemic toxicity. However, complete glycogen depletion was also noted in the liver in one control animal, and the rest of controls (5/6) showed minimal glycogen vacuolation. This effect could be a consequence of fasting and there is thus no apparent reason to disregard the observed DNA effects based on the toxicity observed.

No genotoxicity tests were performed with germ cells. However it seems likely that cholecalciferol and/or its metabolites reach germ cells since vitamin D metabolising enzymes are present in reproductive tissue (discussed in section 9) and vitamin D is suggested to be important for normal male reproductive function. Moreover, testes, mature spermatozoa and the ejaculatory tract express both receptors and the vitamin D metabolising enzymes (section 10.8.1).

A report describing a double-blind randomised clinical trial of oxidative DNA damage is available in the open literature (Fedirko et al., 2010). In this trial, the degree of DNA damage was assessed using 8-hydroxy-2'-deoxyguanosine (8-OH-dG) as a biomarker of oxidative DNA damage in biopsy tissue samples. Vitamin D3 supplementation for 6 months at 800 IU/day (20 µg/day) was associated with a decrease in 8 (OH) dG in biopsy tissue, a difference that appeared statistically significant among the small subset of subjects with higher VDR expression. Slightly less decrease was seen among subjects given calcium but no vitamin D. The effect was not apparent in subjects given vitamin D plus calcium. Although this is an interesting result suggesting a protective effect of vitamin D towards oxidative DNA damage, the study does not add to the interpretation of the findings in the standard test battery for genotoxicity. The comet assay detects single or double DNA strand breaks but it cannot be used to clarify the cause of the effects. In the absence of further information, it is not possible to exclude that the positive results obtained in the two studies may result from other modes of action than oxidative DNA damage. The presence of DNA effects in liver that are not seen in the colon may be due to a liver specific metabolite that is not present in colon.

10.8.2 Comparison with the CLP criteria

The criteria in table 3.5.1 of Regulation 1272/2008 for a category 2 mutagen read:

— positive evidence obtained from experiments in mammals and/or in some cases from *in vitro* experiments, obtained from:

— somatic cell mutagenicity tests *in vivo*, in mammals; or

— other *in vivo* somatic cell genotoxicity tests which are supported by positive results from *in vitro* mutagenicity assays.

These criteria are considered fulfilled since cholecalciferol was positive in the comet assay i.e. increased DNA migration in liver cells occurred near or at estimated lethal doses but without any apparent liver tissue damage that could have interfered with the result.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

An additional classification to current classification as Muta 2, H341 is warranted. Nevertheless, there is no concern for germ cell mutagenicity at exposure levels restoring normal vitamin D levels (i.e. exposure in the dietary supplementation range).

10.9 Carcinogenicity

Table 10.9.a: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
26 week study, limited comparability to an OECD 408 guideline study and is not performed in accordance with GLP	Rat, Charles River Crl:CD BR rats, 30/group	C9756	0.125, 0.25, 0.5 mg/kg bw/day	***At 0.125 mg/kg:*** 1/12 proliferative adrenal lesion (hot spot[1]). Stat. significant increase in BrdU labelled chromaffin cells week 4,8,12 but not week 26. Stat. sign. Increased serum calcium and phosphorous and urine calcium/creatinine ratio wk 4-26. No effect on body weight gain, no effect on adrenal weight. ***At 0.25 mg/kg bw:*** ↑ Absolute (wk 12, 26) and relative adrenal weight (wk 12) Adrenal proliferative lesions (hotspots) in 8/10 animals (none in controls). Hyperplastic nodules 5/10, phaeochromocytoma 1/10 ↓ body weight gain approximated to be around 10% lower than mean control body weight at end of study. Reduced	IIIA 6.5-02 Tischler, A., S et al (1999) Toxicol Sci. 1999 Sep;51(1):9-18.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
				weight gain (growth curves followed the control and low dose group until approx. day 77 for 0.25 mg/kg group) Stat. sign. Increased serum calcium and phosphorous and urine calcium/creatinine ratio wk 4-26. Hypercalcaemia (based on serum calcium and urinary calcium excretion, mild nephrocalcinosis without scarring ***At 0.5 mg/kg bw:*** ↑ Abs. (wk 12) and ↑ rel adrenal weight wk12/26: i.e. 22% vs 12% in controls resp. 22% vs 10% in controls. Adrenal proliferative lesions in 9/9 animals –Hotspot 8/9, Hyperplastic nodules 7/9, phaeochromocytoma 1/9 ↓ body weight gain, 30-35% lower than mean control body weight at end of study. Hypercalcinosis based on mild to moderate nephrocalcinosis with scarring. Stat. sign. Increased serum calcium and phosphorous and urine calcium/creatinine ratio wk 4-26 Figures represent differences (in %) to controls	
57 week study in rats limited comparability to an OECD 452 guideline study and is not performed in accordance with GLP	Rat, wistar males 20/group	24, 25-dihydroxycholecalciferol An active form of vitamin D. Unknown purity of test substance.	0 and 5 ppm in the diet	↑ Serum phosphorus ($p < 0.05$) no significant differences to controls in the other serum parameters including calcium. ↑ Urinary calcium levels at all investigated time points ($p<0.05$ or 0.01). Max. at 22 weeks. ↑Urinary phosphorous at 22 weeks only ($p<0.05$). ↑ Absolute adrenal weight approximately 20% (right, $P<0.01$) and 19% (left, $P<0.05$) and relative by 19% (right, $p<0.01$) and 16% (left $p<0.01$). ↑Absolute and relative femur weight by 17% ($p<0.01$) and 19% ($p<0.01$) respectively. ↑Absolute and relative kidney weights (1.5 to 4%) compared to controls but	IIIA 6.5/03

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
				only statistically significant for a 4% ($p<0.05$) relative left kidney weight. ↑ Medullary adrenal hyperplasia 6/20 ↑ phaeochromocytoma in 1/20 rats (mostly ↑PCNA labelling indices in the normal area of the adrenal compared to control animals (1.82 vs 0.87) with increasing indices in the area of medullary hyperplasia (5.88) and in the phaeochromocytoma (16). The cortical bone of femur was also thicker in treated rats. It was specifically stated in the paper that no apparent toxic changes including calcifications was histopathologically recognised in the lungs, heart, liver, kidneys, thyroid, parathyroid, spleen, stomach and intestines.	

[1] Hotspot were defined as loose clusters of 5 or more cell nuclei labelled with the proliferation marker BrdU that stood out sharply against the background population of sparsely marked cells. Hot spots were not readily visible by haematoxylin eosin staining

[2] The increased chromaffin cell proliferation at the low dose were no longer statistically significant at the end of study whereas consistently increased to end of study at higher doses.

Table 10.9.b: Summary table of human data on carcinogenicity

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
EFSA Scientific Opinion	NA	Type: Opinion of the Scientific Committee/ Scientific Panel On request from: European Commission Question number: EFSA-Q-2011-00955 Adopted: 26 June 2012 Published: 27 July 2012 Affiliation: European Food Safety Authority (EFSA), Parma, Italy	Hypercalcaemia and hypercalciuria was assessed in studies using daily or weekly supplementation of vitamin D for several weeks to months with an aim to derive a tolerable upper intake level in humans. Long-term health outcomes (all-cause mortality, cardiovascular disease, cancer, fractures and kidney stones) were also considered. Fertility was not specifically addressed. But pregnant and lactating women were considered. . In adults, a daily vitamin D dose of 250 µg/day (range 234-275 µg/day) was considered to reflect a no observed adverse effect level (NOAEL). This value was based	EFSA Journal 2012;10(7):2813 http://www.efsa.europa.eu/en/efsajournal/pub/2813.htm

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
			on only two studies of short duration (up to five months) in small samples of healthy young men with minimal sun exposure. To take into account the uncertainties associated with this value, an uncertainty factor of 2.5 was chosen, and a tolerable upper intake level (UL) for adults (also applicable to pregnant and lactating women) was established at 100 µg/day. This UL was supported by two studies in pregnant and lactating women, both using doses of vitamin D2 or D3 up to 100 µg/day for several weeks to months, which did not report adverse events for either the mothers or their offspring Cancer: No increased risk in the dose range 10 to 27.5 µg/day for 4 to 7 years when reviewing information from several clinical trials. Breast and colon cancer was the secondary outcome in these trials. A meta-analysis of observational studies published up to 2011 found no association between 25-hydroxycholecalciferol concentration and breast cancer (5 studies) or prostate cancer (11 studies). Inverse correlation was found with colorectal cancer (9 studies). Inconsistency in reported associations between 25-hydroxycholecalciferol and cancer. In one observational study there was a significant increase in total cancer mortality in elderly Swedish men with base line serum 25-hydroxycholecalciferol at > 98 nmol/L (but not> 93 nmol/L). In one cohort study there were significant increase in total cancer mortality in US men (but not women) with baseline 25-hydroxycholecalciferol in the two categories 80-<100 nmol/L and ≥ 100 nmol/L compared to men with 25-hydroxycholecalciferol concentration <37.5 nmol/L (overall trend was not significant). Four other cohort studies did not report any association. Inconsistent associations of vitamin D and 25-hydroxycholecalciferol concentration and pancreatic cancer was also reported by EFSA. A summary and discussion on the data reviewed by EFSA and relevance for hazard assessment is provided in section 10.9.1 below	
Systematic Literature	NA	Published: Vitamin D – a systematic literature	**Cancer** Vitamin D and cancer have been studied in	IIIA6.12.4-02

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
review (SLR)		review for the 5th edition of the Nordic Nutrition Recommendations A systematic review of literature on effects of Vitamin D is reported. The review was intended to update existing Nordic Nutritional Requirements, in particular where new scientific information has emerged. The research questons were the following: "*1. What is the effect of vitamin D from different sources on serum 25(OH)D concentrations?* *2.What is the relationship between 25(OH)D concentrations and different outcomes in different populations and age groups?* *3.What is the effect of dietary vitamin D intake on different outcomes in different populations and age groups?* *4.What is the effect of supplemental vitamin D on different outcomes in different populations and age groups?* *5.What is the effect of sun or UVB exposure on different outcomes in different populations and age groups?* *6.What is the UL (tolerable upper intake level) for vitamin D for different health outcomes in different populations and age groups?* *7.What are the interactions of vitamin D with calcium intake on different health outcomes in different populations and age groups?* *8.Which is the interaction of vitamin D intake or vitamin D status with vitamin A intake or vitamin A status on health outcomes in different populations and age groups?"*	several cohort studies. Some randomized clinical trials (RCTs) have been performed as secondary analyses in studies for the prevention of fractures. Total cancer: There was not consistent evidence for an association between vitamin D status and total cancer in SLRs including cohort studies and RCTs. Colorectal cancer: There is some observational evidence of an inverse association between vitamin D status and risk of colorectal cancer, however evidence for a causal relationship was lacking. Breast cancer: The evidence for an inverse association between vitamin D status and breast cancer risk was weak due to lack of good quality studies and heterogeneity between studies. Prostate cancer: There was little or no evidence for a protective effect of vitamin D on prostate cancer. In the overall discussion, the authors note that the observation that S-25-hydroxycholecalciferol is inversely related to some types of cancer **Total Mortality** It is concluded that vitamin D3 (10-20 µg/day) combined with calcium significantly reduces total mortality. The effect of cosupplementation with calcium was unclear. A Swedish cohort study among elderly men followed for around 14 years reported increased all-cause mortality both in men at the low (<46 nmol/L) and the high end of 25-hydroxycholecalciferol concentrations (>98 nmol/L). Another Danish cohort study found a reverse J-shaped relationship with lowest mortality at 25(OH)D concentrations of 50-60 nmol/L.	
Randomized, placebo-controlled	CaD (1 g Ca/400 IU vitamin D	Published Calcium and vitamin D supplements and health outcomes: a re-analysis of	The study give some support to that vitamin D/calcium supplementation can have a beneficial effect on individuals that did not take personal supplements at randomization.	IIIA6.12.4-03

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
trial (7-years)	daily)	the Women's Health Initiative limited-access data set	However it cannot be determined on the basis of this study whether vitamin D, calcium or the combination is attributed to the effects. The reliability of the results is also lowered by the study being a post hoc adjusted analysis. The study concluded a beneficial effect for those without previous supplementation of the added 400 IU on total cancer, and total breast cancer (and an effect without statistical significance on colorectal cancer). The study authors also suggested that the beneficial effects on these cancers were threshold related rather than dose response related since no further decrease in cancer incidence was found in the already supplemented dose group. It should be noted that the two groups (without personal supplements or taking personal supplements at randomization) differed at randomization in several factors associated with co-morbidity (age, BMI, blood pressure, hormone replacement therapy, smoking status and history of myocardial infarction, stroke fracture or diabetes). The study hypothesis was based on a previous re-analysis of the same dataset for another outcome, cardiovascular effects. The authors tried to minimize the risk of over interpretation of the findings and false-positive results by estimating the likelihood of a false positive test by performing eight interaction tests. They estimated that if the effect were unrelated to personal calcium or vitamin D use, and the endpoint were independent, the probability of at least one false-positive interaction test was 33%.	

Table 10.9.c: Summary table of other studies relevant for carcinogenicity

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference

Gavage, 90 day study, rats, OECD408	D0074	Reliability 1	***At 0.06 mg/kg bw/day:*** ↑Hypercalcaemia [serum Ca] ↓ Urinary Ph tissue mineralisation (kidney, trachea) Kidney changes (degeneration/regeneration, tubule dilatation) ***At 0.3 mg/kg bw/day:*** ↑Hypercalcaemia [serum Ca] ↑hyper-phosphataemia [serum P] ↓ Urinary Ph tissue mineralisation (aorta, heart, kidney, stomach, trachea); associated kidney (weight, degeneration/regeneration, proteinacheous casts, tubule dilatation) and adrenal changes (weight, chromaffin cell hyperplasia) Mortality 1/10 males See table 10.9.d for details.	IIIA 6.4.1/01

Table 10.9.d: Summary table of effects observed in the 90 day rat study (mean % difference from controls males/females)

	Mean % difference from controls males/females		
Dosage (mg/kg/day)	**0.012**	**0.06**	**0.3**
Mortality	0%/0%	0%/0%	10%/0%
Terminal body weight (g)	-2/5	-3/1	-9/0
Cumulative body weight gain (g)	-3/17	-5/9	-16/-1
Clinical chemistry			
Calcium concentration (mmol/L)	0.4/-0.4	**4**/4**	**21**/15****
Phosphorus concentration (mmol/L)	5/-12	3/11	**16**/12***
Cholesterol concentration (mmol/L)	9/NS	10/NS	**22*/NS**
Urea concentration (mmol/L)	NS/4	NS/1	**NS/21**
Creatinine concentration (µmol/L)	NS/1	NS/0	**NS/19***
Albumin concentration (g/L)	NS/-1	NS/-2	**NS/-9****
Sodium concentration (mol/L)	NS/-0.5	NS/-0.1	**NS/-1.4****
Chloride concentration (mol/L)	0.7/-0.1	0.4/-0.4	**-2*/-3****
Triglycerides concentration (mmol/L)	1/NS	-6/NS	**15/NS**
Urinalysis			
pH value	4/0	**-13**/-8***	**-9*/-5**
Organ weights			
Adrenal glands			
Absolute (mg)	5/-1	-1/8	**25**/23****
Relative to body weight	9/-6	3/6	**38**/20****
Relative to brain weight	6/-4	-2/7	**27**/21****
Testes			
Absolute (g)	-8/NR	-9/NR	**-11*/NR**
Relative to body weight	-5/NR	-5/NR	-2/NR
Relative to brain weight	-7/NR	-10/NR	**-10*/NR**
Seminal vesicles			
Absolute (g)	1/NR	-5/NR	**-16**/NR**
Relative to body weight	5/NR	0/NR	-7/NR
Relative to brain weight	3/NR	-4/NR	**-14*/NR**
Kidney			
Absolute (g)	NS/4	NS/2	**NS/32****
Relative to body weight	0.4/-2	1/0	**9*/30****
Relative to brain weight	NS/0	NS/1	**NS/30****
Ovary			
Absolute (g)	NR/12	NR/13	**NR/33**

	Relative to body weight	NR/6	NR/12	**NR/33***
	Relative to brain weight	NR/8	NR/12	**NR/32**
Microscopic observations				
Dosage (mg/kg/day)	**0**	**0.012**	**0.06**	**0.3**
Number of animals examined	10/9	10/10	10/10	9/10
Aorta abdominal Mineralisation	0/0	-/-	0/0	**3/4**
Aorta thoracic Mineralisation	0/0	-/-	0/0	**1/3**
Heart Mineralisation	0/0	-/-	0/0	**5/5**
Kidneys Tubular mineralisation	0/0	0/0	**0/1**	**8/6**
Degeneration/regeneration	0/0	0/0	**2/0**	**6/10**
Tubular dilation	0/0	0/0	**2/1**	**5/10**
Cast proteinaceous	1/0	1/0	1/0	**3/0**
Stomach Mineralisation	0/0	-/-	0/0	**1/0**
Necrosis	0/0	-/-	0/0	**1/0**
Trachea Mineralisation	0/0	0/0	**1/0**	**8/6**
Femur Hyperostosis	0/0	-/-	0/0	**4/9**
Sternum Hyperostosis	0/0	-/-	0/0	**8/8**
Adrenal glands Medullar chromaffin cell hyperplasia	0/0	-/-	0/0	**3/4**

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Animal data

The 26-week study in rats is a published study performed with a special focus on adrenal changes. The study was not performed in accordance with GLP and the comparability to an OECD 408 guideline study is limited since only selected parameters were studied. The authors concluded that Vitamin D3 is a powerful and substantial mitogen for adrenal medullary chromaffin cells in male rats and focal proliferative lesions including hot spots and hyperplastic nodules. Phaeochromocytoma, a neuroendocrine tumour secreting high amount of cathecolamines, was observed in 1/10 animals at 0.25 mg/kg bw/day and 1/9 animals at 0.5 mg/kg bw/day. The serum calcium and phosphor levels as well as the calcium creatinine ratio were statistically significantly increased from week 4. Calcification, graded "none to minimal", was observed by week 4 in the kidneys of animals administered 0.125 and 0.250 mg/kg bw/day. The calcinosis was mild at 0.250 mg/day by week 26 with little or no scarring. Animals in the low dose group (0.125 mg/kg bw/day) had normal or minimal calcifications. The kidney calcification was also minimal by week 4 in 3/6 animals of the high dose group (0.5 mg/day) but had progressed by week 26 to "mild to moderate"

nephrocalcinosis with patchy tubular athrophy and scarring. The body weights were only reduced at week 4 in animals administered 0.250 mg/kg bw. The body weights deviated from the control- and low dose weight curve around day 77 and were continuously reduced until week 26. The adrenal weights were increased from week 12 in the 0.250 mg/kg bw and 0.5 mg/kg bw groups. Loss of animals due to premature deaths or euthanasia occurred (i.e. 3 animals at 0.5 mg/kg bw, 2 animals at 0.250 mg/kg bw and one control) but the cause was not reported. Nevertheless, it was noted that there were no detectable abnormalities on adrenal glands in these animals.

Human data

Some information on human exposure to cholecalciferol is available in the scientific opinion prepared by EFSA and in a published systematic literature review of vitamin D for the 5th edition of the Nordic Nutrition Recommendations (the NNR5 project, IIIA 6.12.4-02). Based on the human studies included, these reviews do not suggest an association between exposure and cancer at doses comparable with or slightly above the supplement range. The dose range investigated in these studies were 10 to 27.5 µg/day for 4 to 7 years. There were also no clear association between exposure to 25-hydroxycholecalciferol concentration and cancer. The reports were inconsistent with respect to any cancer association between low- (deficiency) and high 25-hydroxycholecalciferol levels.

EFSA found no increased risk from exposure in the dose range 10 to 27.5 µg/day for 4 to 7 years. Breast and colon cancer were the secondary outcomes in these trials. In addition a reviewed meta-analysis of observational studies published up to 2011 found no association between 25-hydroxycholecalciferol concentration and breast cancer (5 studies) or prostate cancer (11 studies). Inverse correlation was found with colorectal cancer (9 studies). The EFSA panel also reported an inconsistency between the reported associations for 25-hydroxycholecalciferol and all-cause mortality or cancer. The panel reviewed data from one observational study cohort studies. In the observational study there was a significant increase in total cancer mortality in elderly Swedish men with base line serum 25-hydroxycholecalciferol at > 98 nmol/L (but not> 93 nmol/L). In the cohort study there was a significant increase in total cancer mortality in US men (but not women) with baseline 25-hydroxycholecalciferol in the two categories 80-<100 nmol/L and ≥ 100 nmol/L compared to men with 25-hydroxycholecalciferol concentration <37.5 nmol/L (overall trend was not significant). Four other cohort studies did not report any association. Inconsistent associations of vitamin D and 25-hydroxycholecalciferol concentration and pancreatic cancer was also reported by EFSA.

The NNR5 review found four systematic literature reviews on vitamin D and cancer that met their inclusion criteria. These systematic literature reviews included cohort studies and randomised clinical trials. The authors found no consistent evidence for an association between vitamin D status and total cancer. The authors found some observational evidence of an inverse association between vitamin D status and risk of colorectal cancer but there was no evidence for a causal relationship. The evidence for an inverse association between vitamin D status and breast cancer risk was also considered weak in the NNR5 review due to lack of good quality studies and heterogeneity between studies. There is, according to the study authors, little or no evidence for a protective effect of vitamin D on prostate cancer. The authors note that the observation that serum 25-hydroxycholecalciferol is inversely related to some types of cancer is supported by a new reanalysis of a subgroup of participants in the large Calcium and vitamin D (CaD) trial in the Women's Health Initiative (WHI). The WHI CaD was a seven year, randomized, placebo-controlled trial of "CaD" (1 g $CaCO_3$ and 400 IU vitamin D (approximately 10 µg) daily) in 36282 community-dwelling, postmenopausal women. The conclusion from this study was that daily supplementation of calcium with vitamin D for seven years had no effect on the incidence of colorectal cancer among

postmenopausal women. However, it was recognized that since colorectal cancer has a long latency (approximately 10-20 years), the lack of effects may be due to the duration being limited to 7 years.

Relevance to humans

a) Background frequency:

There is a high background frequency of adrenal phaeochromocytomas in male rats. Phaeochromocytoma occur spontaneously, mostly in older rats with an age of > 80 weeks (Greim *et al*. 2009). According to this publication, the frequency varies depending on species, strain, sex and age. The frequency is generally higher in males than females and is reported to be 9-20% in males and 2-6.2% in females for Sprague-Dawley and approximately 10% and 2% in Wistar males and females after 24 months. The frequency has also increased over time. However, the published historical control data in older rats is less relevant for the rat study since the tumours were observed already after 26 weeks. The lack of relevant historical control data is thus very unfortunate and complicates the assessment of the relevance of findings. Moreover, female rats were not included in the 26 week study but increased proliferation in adrenals was observed in female rats in the 90 day study (table 10.9.d). Phaeochromocytoma is a rare tumour in humans with an annual frequency of 2-8 cases per million.

b) Mechanism of tumor formation

Tischler et al. (1996) found that vitamin D was a mitogen *in vivo* but not *in vitro*. It has therefore been suggested that the vitamin D effect is a consequence of impaired calcium homeostasis by increased calcium absorption (Tischler et al., 1996, IIIA 6.5-02). The adrenal tumors occurred at the same doses as increased serum calcium and nephrocalcinosis. In another study, adrenal cell proliferation was stimulated by hypocalcemia in rats when induced by intravenous infusion of calcium gluconate (Isobe et, al., 2012). On the other hand, phaeochromocytoma was also observed in a study in Wistar rats with a vitamin D metabolite that did not increase the serum calcium (IIIA.6.5-03). In this study medullary hyperplasia and phaeochromocytoma occurred in adrenals in 1/20 animals at 57 weeks, when 24R, 25 dihydroxyvitamin D3 was given. The males studied had increased urinary calcium whereas the serum calcium was not increased. The femur and adrenal weight was increased. The serum calcium was only investigated at study termination. The study was not performed according to the principles of GLP or OECD test guidelines and the results are not considered to bring further clarity to the mechanism for phaeochromocytoma formation.

c) Relevance to humans

The authors of the 26-week study underline that there is no evidence that hypercalcaemia is associated with phaeochromocytomas in humans however this statement is not supported by any large scale epidemiology data. The authors found cases of phaeochromocytoma to be chance findings in patients with sarcoidosis. Hypercalcemia and hypercalciuria can occur in sarcoidosis patients. A case study was presented to support the conclusion but this report concluded that there were few data to support a causal relationship between sarcoidosis and phaeochromocytoma and the probability for a chance based association was instead estimated. Hyperparathyroidism was, according to the study authors, probably also not associated with phaeochromocytomas, except in patients with multiple endocrine neoplasia syndromes. No reference was given to the statement. Overall, it is not considered possible to conclude, based on this information, if there may be an association between hypercalcemia and phaeochromocytoma in humans. In addition, information is lacking with respect to any association of high dose (i.e. doses outside the supplement range) exposure to vitamin D in humans and cancer. The human data described above only support lack of association with cancer in the dose range 10 to 27.5 µg/day. The data on high plasma concentration of 25-hydroxycholecalciferol were inconsistent and cannot be used to clarify the question.

Greim *et.al*. (2009) investigated the relevance of mechanisms of chemically induced phaeochromocytomas for human risk assessment.
They reviewed the occurrence of phaeochromocytomas in animal studies directly or indirectly induced by chemicals and found that phaeochromocytomas are often found in test animals in addition to other tumors (especially in male rats). They listed involvement of conditions such as hypoxia i.e. impaired respiration or pulmonary toxicity, uncoupling of oxidative phosphorylation, disturbances in calcium homeostasis e.g. kidney damage that lead to disturbed calcium secretion via urine and disturbance of the hypothalamic endocrine axis. In addition they suggested that interference with biochemical mechanisms lead to phaeochromocytoma and especially via interference with enzymes in catecholamine synthesis, receptor tyrosine kinase, hypoxia inducible factor and fumarate hydratase. Although the mechanism of action identified in rats is expected in humans the authors did not find evidence to support that substances producing phaeochromocytoma in animals also induced corresponding tumours in humans. However, they state that the evidence was inadequate for a final conclusion.

With respect to classification, the CLP guidance section 3.6.2.3.2 only lists phaeochromacytoma arising in male rats exposed to particulates through inhalation (secondary to hypoxemia) as a mechanism that is not relevant to humans.

Table 10.9.e: Compilation of factors to be taken into consideration in the hazard assessment

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
26 week study	Phaeochromo-cytoma 1/10 (10%) males No historical data available for the actual study and the historical data relate to published information in 1968-1992. Frequency in SD rats (and in rats in general) seems to increase with age in the rat and over time (Greim et. al. 2009).	Not investigated (but positive comet assay for genotoxicity).	Adrenal proliferative response (chromaffin cells) in 90 day study (males and females) and in the 26 week study (males only)	Phaeochromo-cytoma already at 26 weeks	Only males investigated in the 26 week study but the adrenal chromaffin cell proliferation was also increased in females in a 90 day study	Phaeochromocytoma at the same dose as hypercalemia (serum calcium and urinary calcium excretion, mild to moderate nephrocalcinosis without scarring) reduced body weights approximated to be around 10%. But at the next dose 0.25 vs 0.5 µg/kg bw/day the toxicity was excessive with decreased body weight gain already week 1 and 30-35% lower mean body weight than controls at end. Scarring also occurred in kidneys at this doe. Unexplained deaths occurred in study but no effect on adrenal glands reported for these animals.	oral	Hypothesis that vitamin D induce chromaffin cell proliferation in the adrenal medulla of rats due to impaired calcium homeostasis. Vitamin D regulate calcium homeostasis also in humans. Human data negative for carcinogenicity in the supplement range. No positive human data at higher doses but data consists mostly of case reports and from treatment of various diseases or together with other substances. Inconclusive data in the literature reviewed by EFSA with respect to any association between increased cancer risk and 25-hydroxycholecalciferol concentration

10.9.2 Comparison with the CLP criteria

Category 1A

According to CLP criteria, classification in category 1A is appropriate if the substance is known to have carcinogenic potential in humans, principally based on human evidence.

The published literature data available do not demonstrate an association between tumour cases and exposure to Vitamin D3 in the range used for dietary supplementation. However, there is limited information on effects from high doses of cholecalciferol (outside the supplement range) after prolonged use in humans. Therefore, data is considered insufficient to support classification in this category.

Category 1B

A substance should be classified in Category 1B if a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of a combination of benign and malignant neoplasms in at least two species or in two independent studies in one species.

The only animal data available include a 90 day study in Wistar rats, a 26 week study in Charles River rats and a 57 week study in Wistar rats (performed with 24, 25-dihydroxycholecalciferol). Some effects could be considered to indicate a carcinogenic potential, i.e. cell hyperplasia in adrenal glands in the 90 day study, hotspots in adrenals and phaeochromocytoma in the 26 week study, hyperplasia in adrenal glands and phaeochromocytoma in the 57 week study. Unfortunately, the study durations were in all cases too short to represent the life-time of the rat and considering the tumour latency, it cannot be concluded from these results if other tumour types would develop. Therefore data from experimental animals is considered insufficient to support classification in category 1B.

Category 2

The CLP regulation states in section 3.6 (table 3.6.1):

"*The placing of a substance in Category 2 is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations (see section 3.6.2.2 of the CLP). Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies*".

and furthermore:

"Limited evidence of carcinogenicity"- the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g.
(a) the evidence of carcinogenicity is restricted to a single experiment;
(b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies;
(c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or
(d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs."

The 26 week study is considered to provide limited evidence of carcinogenicity based on criteria (a) and (d) above: Progressive lesions were observed in rats since proliferation increased in chromaffin cells of the medulla and progressed to phaeochromocytoma in 1/9 animals at the medium and high dose. The adrenal weights were correspondingly increased.

- *(a) tumour type and background incidence;*

Phaeochromocytoma was observed in 1/10 (10%) and 1/9 (11%) animals administered 0.25 or 0.5 mg/kg bw/d for 26 weeks. This type of tumour is relatively common in aging male rats but in this study it was observed already after 26 weeks. There is no relevant historical data available (i.e. data considered relevant for the time of study, laboratory, age of rats etc).

- *(b) multi-site responses;*

There are no studies available covering the lifespan of the test species. Therefore, taking into account tumour latency, it is not possible to conclude on this parameter. However, the positive result obtained in the *in vivo* comet assay raises some concern for carcinogenicity via a genotoxic mode of action.

- *(c) progression of lesions to malignancy;*

90 day study: Adrenal proliferative changes were observed in chromaffin cells of the adrenals in 3/9 male and 4/10 female rats administered 0.3 mg cholecalciferol/khg bw/d.

26 week study: Hot spots in adrenals (i.e. loose clusters of ≥5 labelled cell nuclei) were observed in 1/12, 8/10 and 9/10 (with hyperplastic nodules in 7/9) animals administered 0.125, 0.25 and 0.5 mg/kg bw/d.

57 week study: Medullary hyperplasia and phaeochromocytoma were observed in 6/20 and 1/20 animals administered 5 ppm of 24, 25-dihydroxycholecalciferol (a cholecalciferol metabolite).

- *(d) reduced tumour latency;*

There is no data available covering the entire life-span of the test species but the phaeochromocytomas were observed already after 26 weeks.

- *(e) whether responses are in single or both sexes;*

Only males were included in the two studies where phaeochromocytomas were observed (26 and 57 week studies). However, adrenal chromaffin cell proliferation was observed also in females in the 90 day stduy.

- *(f) whether responses are in a single species or several species;*

The only studies available that are considered to be of relevance for this endpoint were conducted in rats.

- *(g) structural similarity to a substance(s) for which there is good evidence of carcinogenicity;*

No information available.

- *(h) routes of exposure;*

All studies available that are considered relevant for this endpoint were conducted via the oral route thus no conclusion with respect to route-specific effects can be made.

- *(i) comparison of absorption, distribution, metabolism and excretion between test animals and humans;*

The vitamin D system with receptors (VDR) and vitamin D-metabolising enzymes are widespread in the tissues of the human body and among vertebrates. Therefore, the effects of cholecalciferol are expected to be similar between both humans and vertebrates.

- *(j) the possibility of a confounding effect of excessive toxicity at test doses;*

In the 26 week study, the body weight gain was decreased at the LOAEL (by 10% if approximated from the figure in the publication). Additional toxicity in the form of hypercalcemia indicated by increased serum and urinary calcium levels was described with observations of mild nephrocalcinosis but without scarring. Serum phosphorous was also correspondingly increased. The toxicity was more pronounced at the high dose of 0.5 mg/kg bw/day including more profound body weight reductions, moderate nephrocalcinosis with patchy tubular athrophy and scarring. Unexplained mortality occurred at 0.5 mg/kg bw (3/30 animals), 0.250 (2/30 animals) and in controls (1/30). Nevertheless, there were no abnormalities in the adrenal glands of these animals. Adrenal cell proliferation was an early event occurring before kidney damage and body weight reductions.

- *(k) mode of action and its relevance for humans, such as cytotoxicity with growth stimulation, mitogenesis, immunosuppression, mutagenicity*

The mode of action has not been fully elucidated but it has been suggested that hypercalcemia could cause the tumours observed. However, since cholecalciferol was positive in an *in vivo* comet assay, a genotoxic mode of action cannot be excluded.

In summary, the animal data available indicate that cholecalciferol causes proliferative changes in adrenals of rats and phaeochromocytoma manifest in rats already after 26 weeks of exposure. Since there are no studies available with a duration covering the entire life-span of the test species, it cannot be excluded that tumours with a latency of more than 26 weeks may develop. The mode of action of the phaeochromocytoma observed cannot be established from the data available but taking into account the positive result obtained in the in vivo comet assay, a genotoxic mode of action cannot be excluded. The relevance of this finding to humans is unclear but based on the limited human data available in the open literature (Summarised in the EFSA scientific opinion and in the systematic literature review made by NNR5) there is no association between cancer and exposure to doses of cholecalciferol in the supplement range. Any association at levels outside of this range in humans is unknown.

Conclusion on classification and labelling for carcinogenicity

The data available to assess the intrinsic carcinogenic potential of cholecalciferol is limited. The human data reported is restricted to doses in the range used for vitamin supplementation and the animal data is limited to studies performed with a duration only representing approximately 25% of the lifespan of a rat. Nevertheless, phaeochromocytomas was observed already after 26 weeks and there is thus considered to be evidence from animal studies that high doses of cholecalciferol could be carcinogenic in humans. Therefore, classification in category 2 is proposed. Specific concentration limits are not considered warranted.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 10.10.a: Summary table of animal studies on adverse effects on sexual function and fertility

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Not stated, Not GLP 60 days premating through pregnancy and lactation Investigation of uterus for embryos, implantation , resorptions and corpora lutea im 50% of females of each group at GD13 Reliability 2-3	The main deviations to OEDC 416 guideline were: No info on acclimatisation or previous treatments or age of animals. The number of pregnant animals was 20-24 but only 50% of these were allowed to deliver. The identity of the control solution was not given. Shorter pre mating period in males (60 vs 70 days). No assessment of sperm parameters. Females were treated over at least 2 oestrus cycles. No F2 generation, only F1 offspring studied until day 21 of lactation. There were no details on clinical observations given (nor if it was performed and how often). Body weights seem to have been recorded on a weekly basis (it is mentioned that statistics was performed on weekly body weights but results were not tabulated). Sex of pups and body weight of pups was not given in the study, functional observations of pups was not made, terminal	Rat, Crl:CD 20/group	1α,25-Dihydroxyvitamin D3 (Calcitriol) Rocaltrol®, Roche	0, 0.02, 0.08, 0.3 µg/kg bw/day Gavage	**Parental:** **0.02 µg/kg/day:** ↓ Serum phosphate (-10%) **0.08 µg/kg bw:** ↓ Serum phosphate (-11%) **0.3 µg/kg bw:** ↓ Serum phosphate (-13%), ↑ Serum Calcium (11%) **Offspring:** **0.02 µg/kg/day:** ↑Urea nitrogen (25%) **0.08 µg/kg bw:** ↓ Serum phosphate (-6%) **0.3 µg/kg bw:** no stat. sign. changes in serum chemistry No stat sign. Changes in bone ash, No effect on % pregnant, average litter size, average	IIIA6.8.2-02 McClain RM, *et al* (1980) Toxicol Appl Pharmacol. Jan;52(1):89-98

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
	organ or body weights was not given, histopathology of reproductive or known target organs was not performed				no. of implantation sites/litter, % resorptions, average no. corpora lutea/litter, gestation-, viability-, lactation index.	

Table 10.10.b: Summary table of human data on adverse effects on sexual function and fertility

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
EFSA Sci. opinion		Fertility was not specifically investigated but an UL was set that was set for all age groups (life-stages).		See table 10.9.b
Systematic Literature Review (NNR5 project)		Fertility was not specifically investigated.		See table 10.9.b
Systematic Literature Review (Published)		A systematic literature search in Pubmed of English language publications until October 2011. The search term used in the review were: vitamin D and fertility, vitamin D and reproduction, vitamin D and PCOS and 25-hydroxyvitamin D (25(OH)D, 1,25-dihydroxyvitamin D and calcitriol as alternatives to vitamin D. The review did not include information on inclusion basis of the selected papers (if all were included or on what basis some	The review investigated both animal and human study data (both on women and men). Some evidence for involvement of vitamin D on reproduction was found (mostly from animal studies).The authors suggested that there were some evidence for involvement of vitamin D on polycystic ovarian syndrome (PCOS) and IVF outcome. Some evidence in men was suggested on vitamin D involvement on semen quality and androgen status. The evidence for effects on on fertility was mostly directed at the risks of	Lerchbaum E and Obermayer-Pietsch B., 2012

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
		were selected as relevant). Nor were quality parameters of the reviewed papers included.	fertility effects by vitamin D deficiency. In general the human data were based on observational studies and generally inconsistent. The authors concluded that high-quality RCTs with a large sample size would be needed to determine optimal 25(OH)D levels and also to evaluate the effects of vitamin D supplementation on fertility. Some evidence were for example found (observational studies) on semen quality and vitamin D but no RCTs were found that investigated this effect.	

Table 10.10.c: Summary table of other studies relevant for toxicity on sexual function and fertility

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Review	Not Applicable	Not Applicable	The vitamin D metabolising enzymes and VDR receptors has been identified in the male reproductive tract and in sperm in tissue samples from humans	IIIA.6.14
Historical Synopsis submitted by the biocides applicant	Not Applicable	Not Applicable	Eleven studies between 1961 to 1996 were reviewed for effects on fertility. Five of these indicated effects on fertility but are excluded as relevant for classification based on the following information from the synopsis: -Administration of the substance s.c. or i.v. -High doses in the range of lethality were given.	IIIA.6.8.1-07

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

Animal data

The reproductive and developmental toxicity of metabolite calcitriol (1α, 25-Dihydroxycholecalciferol) in rats and rabbits was investigated in a study which is described in an old publication from 1980. The results from this study that are considered relevant for sexual function and fertility are discussed below whereas results relevant for developmental toxicity are discussed in section 10.10.4. Unfortunately, the study lacks several analyses that are important for determining reproductive effects. Some of the main deviations were the following:

- only 10-12 animals were allowed to deliver since 50% of the mothers were terminated on gestation day 13 for investigations of uterus (for embryos, implantation, resorptions and corpora lutea)
- the pre-mating period was slightly shorter in males (60 vs the recommended 70 days)
- sperm parameters were not investigated
- the offspring was only studied until day 21 of lactation thus the study did not include an F2 generation
- histopathology on known target organs or reproductive organs was not performed
- the information on maternal toxicity was only reported as a dose-dependent hypercalcemia and hypophosphatemia based on serum chemistry data

Besides the limited investigation of reproductive effects, further shortcomings of the study include that it is performed with a metabolite rather than cholecalciferol and that it is not clear if the doses used were close to the maximum tolerable dose in the rats.

The results in dams showed an increased serum calcium level at 0.3 mg/kg bw (high dose) whereas phosphate levels decreased at all doses tested. There were no effects with respect to the percentage pregnant, average litter size day 13, number of implantations, percentage resorptions, average number of corpora lutea per litter, average litter size at birth, gestation index, viability index day 4 and lactation index day 21. In a part of the study effects on late gestation and during lactation were investigated. The dosing in this part of the study was between GD 15 and lactation day 21. The analyses showed increased serum calcium in the offspring but statistical significance was only reached at 0.08 (mid dose) and 0.3 µg/kg bw/day (high dose). The serum calcium was also increased in the mothers at the same doses.

No structural effects on reproductive organs were observed in the 90 day study in rats (section 10.9).

The biocides applicant also provided a summary briefly describing a number of studies investigating effects of high doses of vitamin D (IIIA.6.8.1-07). According to this information five of the eleven studies indicated some effects on fertility. However, two of the studies indicating effects may be excluded since the first study investigated only high doses (2500 µg/kg bw) and doses of 2.5-250 mg/day administered subcutaneously (Mizrahosseini *et al*., 1996) and the second study investigated effects of an intravenous dose of 0.45 µg/kg bw calcitriol (Latorre, 1961, Horii et. al.1992). None of these administration routes are considered

relevant for this assessment. Two other studies may also be excluded since they investigated very high doses where lethality was present or was expected (i.e. 3750-5000 µg/kg bw in Nebel and Ornstein 1996 and 1-3 mg/kg bw in Sharma et. al., 1972). These studies were available when cholecalciferol was discussed for effects on fertility and development by the Commission Working Group on Classification and Labelling of Dangerous Substances and by the Specialised Experts (see part A, section 3).

Human data

Neither the EFSA scientific opinion nor the Nordic Nutrition Recommendations (NNR5 project) specifically addressed fertility effects. In addition to these reviews, the dossier submitter has noted a recent review on human and animal data which includes a discussion on the involvement of vitamin D on fertility. This review found some evidence that vitamin D is involved in fertility but mostly in relation to deficiency (Lerchbaum et. al 2012). The study authors of this review concluded that there is a lack of human studies.

According to reviews, vitamin D is proposed to be important for normal reproductive function (Blomberg-Jensen M. 2012). This is supported by the fact that vitamin D metabolising enzymes and the vitamin D receptors have been identified in the male reproductive tract (sperm and tissue samples) from humans (Blomberg JM, *et al* (2010).

10.10.3 Comparison with the CLP criteria

The data considered for the assessment of possible effects on fertility include:

- A non-GLP fertility study performed with metabolite calcitriol in rats.
- A systematic literature review by Lerchbaum E. and Obermayer-Pietsch, B., 2012.
- A historical synopsis of 11 studies (9 acceptable) provided by the applicant for the assessment under BPD/BPR.

According to 1272/2008, the criteria for classification in Category 1A or 1B are considered fulfilled when a substance is known to have produced an adverse effect on sexual function and fertility in humans, or when there is evidence from animal studies to provide a strong presumption that the substance has capacity to interfere with reproduction in humans.

The criteria for a classification in Category 2 reads (table 3.7.1(a) of the CLP Regulation):

Suspected human reproductive toxicant

"Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects"

There were no effects on the fertility parameters or on sexual function observed in the study performed with metabolite calcitriol or in the 90 day study described in section 10.9.

However there is no robust information available, neither for humans nor for animals, in which effects on fertility parameters have been carefully investigated in a relevant dose range. Therefore, the data on cholecalciferol is not considered sufficient to allow for an accurate assessment of whether or not the substance has intrinsic properties meeting criteria for classification for reproductive toxicity. It is only possible to conclude that no concern for fertility effects is indicated at exposure levels restoring normal essential vitamin D levels (supplement range).

Consequently, no classification is proposed for effects on fertility.

10.10.4 Adverse effects on development

Table 10.10.d: Summary table of animal studies on adverse effects on development

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Not stated, not GLP Reliability 2-3	The main deviations to OEDC 414 guideline were: -only period of organogenesis -1/3 of foetuses examined for visceral alterations. - No info on acclimatisation, previous treatments or age of animals. -The identity of the control solution was not given. - No details on clinical observations (nor if it was performed and how often). -Body weights seem to have been recorded on a weekly basis but detailed results thereof and maternal weights at sacrifice was not presented (it is mentioned that statistics was performed on weekly body weights but results were not tabulated). -No historical controls -Effects was only given as a total and	Charles River CD rat 20 females/ group	1α,25-Dihydroxyvitamin D3 (Calcitriol), Rocaltrol®, Roche	0, 0.02, 0.08, 0.30 µg/kg bw/day Gestation Day (GD) 7 to 15	**Parental:** **0.02 µg/kg/day**: No stat. sign. changes **0.08 µg/kg bw:** ↓ Serum phosphate (-15%), ↑ Serum Calcium (20%) **0.3 µg/kg bw:** ↓ Serum phosphate (-15%), ↑ Serum Calcium (29%), ↑Urea nitrogen (26%) **Offspring:** **0.02 µg/kg/day**: No stat.sign changes in serum/urinary chemistry **0.08 µg/kg bw:** ↑ Serum Calcium (20%) **0.3 µg/kg bw:** ↑ Serum Calcium (23%), ↓ Serum phosphate (-15%), No stat sign. Changes in bone ash, No effect on % pregnant,	IIIA.6.8.1-02 McClain RM, et al (1980) Toxicol Appl Pharmacol. Jan;52(1):89-98

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
	not tabulated per effect per group. -Sex ratio of pups was not recorded.				average litter size, average no. of implantation sites/litter, average fetal body weight, % resorptions, maternal mortality, % external (0 in all groups)-, visceral (approximately 4% in all groups incl. controls)-, skeletal abnormalities. (1/149 but in 0.02 µg/kg group only),. % foetuses increased in the low and middle dose by 10 and 7% respectively i.e. no dose response.	
Not stated, not GLP Reliability 2-3	The main deviations to OEDC 414 guideline were: Only the period of organogenesis was exposed. The number of pregnant animals was between 14-29. No info on acclimatisation or previous treatments or age of animals. The identity of the control solution was not given. Time of death was not tabulated for the dams. There were no details on clinical observations given (nor if it was performed and how often). Body weights seem to have been recorded on a weekly basis no were maternal weights at sacrifice given (it is mentioned	Rabbit, NZW, 16/ group	1α,25-Dihydroxyvitamin D3 (Calcitriol) Rocaltrol®, Roche	0, 0.02, 0.08, 0.3 µg/kg bw/day 12 days (days 7-18 post coitum)	**<u>Maternal effects:</u>** **0.02 µg/kg bw/day:** maternal mortality (1/16) **0.08 µg/kg bw/day:** Maternal mortality 0/15 **0.3 µg/kg bw/day:** ↑maternal mortality (3/16, 2 of these 3 had focal renal tubular calcification with add.f-focal calc. in lungs in 1/3) ↓bw (statistically significant but no information on magnitude), ↑ increased resorptions (26%, nss[1]) vs 12% in controls and at 0.08 µg/kg	IIIA 6.8.2-02 McClain RM, et al (1980) Toxicol Appl Pharmacol. Jan;52(1):89-98

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
	that statistics was performed on weekly body weights but results were not tabulated). No historical control comparisons were presented. Sex ratio of pups was not recorded. Serum and urinary calcium was not measured.				**Developmental:** **0.02 µg/kg bw/day:** % abnormalities ↓(15% vs 22.7 in controls, nss[1]), % skeletal variations ↑81 vs 68 in controls (nss[1]), ↓24 hr viability index (96.1% vs 98.5 in controls, nss[1]) **0.08 µg/kg bw/day** % abnormalities ↑ (33.9% vs 22.7 % in ctrls, nss[1]), multiple abnormalities in one litter*. % skeletal variations ↑ 75%vs 68% in ctrls (nss[1]), ↓ 24 hr viability index (94.2 vs 98.5, nss[1]) **0.3µg/kg bw/day:** % abnormalities ↓(20.6% vs 22.7% in ctrls, nss[1]), % skeletal variations ↑76% vs 68% in ctrls (nss[1]). ↓ 24hr viability index 76.5 vs 98.5, stat. sign), ↓ average fetal bw (-9% lower than controls, nss[1]), ↓average litter size (5.7 vs 7 in ctrls, nss[1]) *See table 10.10.e	

[1] nss= not statistically significant

Table 10.10.e: List of abnormalities in rabbit offspring from reference IIIA 6.8.1/03

		0 µg/kg bw/day	**0.02 µg/kg bw/day**	**0.08 µg/kg bw/day**	**0.3 µg/kg bw/day**
	Number examined	195	127	109	68
External	**Open eyelids**	0	0	9	6
	Syndactyly	0	0	6	0
	Craniofacial	0	0	0	1
	Tail, agenesis	1	0	0	0
Visceral	**Kidney (agenesis)**	0	1	0	0
	Dilated renal pelvis	1	0	1	0
	Microphtalmia	1 (0.5%)	0	9 (8%)	2 (3%)
Skeletal	**Fontanelle (enlarged/irregular)**	39	14	26	4
	Hole in parietal	0	3	0	0
	Cleft palate	0	0	9	0
	Short longbones	0	0	9	6
	Curvature (paws)	0	0	9	0
	Pes caves	0	0	9	0
	Short ribs	0	0	9	8
	Fused ribs	0	0	0	1
	Fused sternebrae	1	2	10	1
	Fused vertebrae	1	0	0	1
	Mosaic sternebrae	0	0	3	0
	Caudal vertebrae (incomplete ossification)	0	0	5	0

Table 10.10.f: Summary table of human data on adverse effects on development

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
Double-Blind, Randomized Clinical Trial of Safety and Effectiveness	Standard prenatal multivitamin containing 400 IU of Vitamin D3 and an additional Vitamin D3 supplement of 0 IU (placebo), 1600 IU or 3600 IU.	Human, 111-122 per group Pregnancy weeks 12 – term 400, 2000, 4000 IU/day or 10, 50 , 100 µg/day cholecalciferol	Parental: no adverse effect Developmental: no adverse effect	IIIA 6.8.1/ 04 Hollis BW *et al* (2011) J Bone Miner Res. Oct; 26(10): 2341–2357.
Clinical trial-pharmacokinetics (PK) study in pregnant women	Vitamin D3 supplement (Vigantol Oil, Merk KGaA, Germany)	Human, 13 pregnant/ 18 non-pregnant 10 weeks before term 70,000 IU (1.75 mg) single dose	Parental: no adverse effect Developmental: no adverse effect	IIIA 6.8.1/ 05 Roth DE *et al* (2012) Nutr J. 2012 Dec 27;11:114. doi: 10.1186/1475-2891-11-114.

Table 10.10.g: Summary table of other studies relevant for developmental toxicity

Type of study/data	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
EFSA Sci. opinion			Human studies during pregnancy and lactation considered by the EFSA panel: One SLR with six RCTs described, dose 20-30 µg/day with or without calcium: No difference in risk placebo or vit D treatment on pre-eclampsia, nephritic syndrome, stillbirth or neonatal death. One RCT up to 100 µg/day cholecaliferol 12-16 weeks of gestation to delivery. There were no difference in number of pregnancy losses (or gestational age at), mode of delivery, pregnancy duration, birth weight or level of neonatal care at delivery. No adverse event attributed to 25(OH)D serum level of vitamin D. No differences in sera calcium level and urinary calcium/creatinine ratio. One trial on lactating women (N=18) with up to 100µg/day month 1-4 of lactation. No increases serum calcium or calciuria. Infant sera 25(OH)D level increased with increased vitamin intake from human milk. One nested case-control study with U shaped association on small for gestational age at birth and 25(OH)D concentration at first half of pregnancy. But limited confounders taken into account (dietary and lifestyle).	See table 10.9.b for more information
Systematic Literature Review (NNR5 project)			Two SLRs was reported on pregnancy related outcomes One was the De-Regil SLR also included in the EFSA Sci opinion. The other SLR investigated 25(OH)D sera concentration and association with preeclampsia (nested case-control). Low concentrations were associated with risk	See section 10.9.b for additional information.

Table 10.10.h: Summary table of observed effects in a series of published studies in rats and mice.

Reference	Results	Comments
Zane 1976	Pregnancy length was unaffected No deaths attributed to vitamin D, no signs of intoxication Early and late resorptions was unaffected no of live foetuses was comparable (no dead foetuses was observed) GD0-3 malformation incidence was unaffected GD4-7 malformation frequency increased by 60% litter frequency, 3.8% fetal incidence. The most frequent anomaly was microcephaly and other abnormalities included facial, palatial and skeletal malformations. Skeletal investigations found retarded ossifications of sternal centers, digits, vertebrae (mainly sacral). No visceral findings.	Dose: 1250µg ergocalciferol/day Duration of treatment: GD0-3 GD4-7 Animals were killed at GD18 Limitations of investigations: Frequency of abnormalities was not reported, nor were a discussion on historical incidences of the findings included. A limited study of ergocalciferol in mice (Wistar strain albino mice), no guideline, GLP and only selected parameters studied.
Potvliege 1962	Body weight of ergocalciferol exposed gravid rats were >20% reduced when compared to the controls. Body weight of foetuses was also reduced. Parathyroid volume was decreased in both mothers and offspring. There were calcifications in organs at both doses but more pronounced at the high dose. Placenta volume was decreased but damage was only histologically confirmed at the high dose. It was reported that there were no constant relationship between grade of placental injury and litter weight but data that showed this was not presented. Non gravid rats were more sensitive than the gravid rats to the same doses. Deaths occurred in the treated non gravid rats already after 6 days whereas gravid rats survived 9 to 10 days of treatment. The organ calcifications were also more severe in the non-gravid rats.	Dose: 500 µg ergocalciferol/day and 1000 µg ergocalciferol/day Corresponding to approximately. 3 to 6 mg/kg bw/day Duration of treatment: GD 12-21 Limitations of investigations: Gavage occurred under general ether anesthesia A limited study of ergocalciferol in rats (albino rats), no guideline, GLP and only selected parameters studied. Serum calcium was not measured.
Ornoy (Ornstein) et. al., 1968	Serum calcium was only increased in treated nonpregnant animals Fetal weight, bone ash and bone calcium and phosphourus content was decreased at 1000 µg/day. At this dose two dams died and all the pups delivered died shortly after delivery. Reduced wet weight, size and ash content of	Dose: 100 µg ergocalciferol /day, 500 µg ergocalciferol/day and 1000 µg ergocalciferol/day Corresponding to approximately. 0.5, 3 and 6 mg/kg bw/day Duration of treatment: GD 10-21 Limitations of investigations:

	placenta at 500 and 1000 µg/day.	A limited study of ergocalciferol in rats (albino rats), no guideline, GLP and only selected parameters studied.
Ornoy et. al., 1972a	At 1000 µg/day (Wistar albino rats): Foetuses to mothers that lost in average 60g body weight died at delivery or PND1 (controls gained in average 50g and pup weight was 6.3g). The weights of the foetuses were also reduced. Dams that had live offspring lost in average 5g bw and had an average offspring birth weight of 5.6g. No gross malformations were observed. Food consumption was reduced by 60-70% during last week of gestation. Pair fed controls (receiving the same amount ingested) gained weight normally, offspring had normal bones on histological examination. The offspring of Charles river rats treated with 500 µg/day died during or shortly after delivery 100% neonatal death was reported (although details was not presented) and the offspring had a low birth weight (3.2g). The offspring was examined for gross malformations and some (without specifying how many) were dissected according to Wilson's method. Skeletal deformities occurred a few days after birth in offspring of treated rats (dose group or frequency was not given): Kyphoscoliosis, distorted shape of extremities (shorter than normal). Severity increased by age. The skeletal anomalies were confirmed histopathologically. Frequency was not tabulated between dose groups but only the wistar rats treated with 1000µg/day was sequentially investigated until postnatal day 30. The wistar rats were less susceptible. When mothers were more severely affected the foetuses weighed less and the neonatal death rate increased.	Dose: 500 µg ergocalciferol/day and 1000 µg ergocalciferol/day Corresponding to approximately. 3 and 6 mg/kg bw/day Duration of treatment: GD 9-21 Limitations of investigations: The reporting of results were limited A limited study of ergocalciferol in rats, no guideline, GLP and only selected parameters studied. Serum calcium was not investigated.
Ornoy and Nebel 1969	Reduced body weight in exposed pregnant and non pregnant females Weight loss: GD17:- 6% GD19: -17% GD21: -19%	Dose: 1000 µg ergocalciferol/day approximately 4 mg/kg bw/day Duration of treatment: GD9-21 (additional non pregnant females were treated for 12 days). Limitations of investigations Statistical investigations was not presented. A limited study of ergocalciferol in rats (albino rats), no guideline, GLP and only selected

	Nonpregnant -36% Controls gained around 10% bw. Hyprecalcemia and hyperphosphatemia non pregnant, treated GD17 and 19 (but not GD 21), controls GD19 (but haemolysed blood). Bone ash and mineral content unaffected but femur has reduced calcium in nonpregnant animals. Fetal weight 32-48% lower than control bw. Reduced bone weight and ash weight > 39 and 40% respectively and increasing by gestation length. Ash percentage was reduced at day 21 only. Bone epiphyses was unaffected, but diaphysis was shorter with highest values day 19 approximately length 30% of control but less pronounced day 21 where the length was around 65-70% of controls. Structural alterations was confirmed by microscopy	parameters studied
Nebel and Ornoy 1972	Reduced fetal and placental weights, altered placenta structure. Reduced fetal viability	Dose: 500 µg/day and 1000 µg/day Duration of treatment: GD 9-20 Limitations of investigations: A limited study of ergocalciferol in rats (no information on strain), no guideline, GLP and only selected parameters studied, limited reporting of effects.
Ornoy 1971	There were only limited effects investigated of ergocalciferol alone in this study, results were only tabulated for number of foetuses, fetal weight, bone ash weight, mineral content and tibial lenght Some retardation of fetal growth was reported (34% lower weight than control foetuses), bone ash was reduced by 14%, calcium content of ash was not affected, phosphor content of ash was 16% compared to 18% in controls. Proximal and distal epiphysis length of tibia was unaffected whereas the shaft was reduced by 20% compared to the control tibial length.	Dose: 500µg ergocalciferol/day Approximately 2 to 2.5 mg/kg bw/day Duration of treatment: GD 8 -10 to GD 18-22 Limitations of investigations: A limited study of ergocalciferol in rats (Charles River), no guideline, GLP and only selected parameters studied In this study one group was given cortisone acetate i.m , one group ergocalciferol p.o and one group received both treatments (results are only presented here for the ergocalciferol group) The purpose was to study the effect on hypercortisonism on fetuses by simultaneous vitamin D treatment.
Ornoy et. al., 1972b	Similar effects as in the study by Ornoy et. al 1972a above was presented eg. reduced fetal and neonatal weight, skeletal anomalies, thin and short bones and impaired ossification increasing by age.	Dose: 500 µg/day and 1000 µg/day to rats (no information on strain) Duration of treatment: GD 8 or 10 to GD 22 Limitations of investigations: Very limited information on materials and

		methods. For example number treated per group was not given. It was described that various bones were cleaned and examined microscopically without specifying. Number of rats subjected to cesarian section and allowed to deliver was not reported. Results were only qualitatively described results was not given by numbers or tabulated.

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Animal data

Several studies in the open literature reporting developmental effects were discussed by the specialised experts for classification and labelling in year 2000 (ECBI/76/00 - Rev. 3, 30.08.2001; ECBI/59/00 – Add. 1 - Rev. 2, 11.04.2001).

Regarding effects on development, "*The Specialised Experts discussed ergocalciferol and colecalciferol in parallel. The Specialised Experts noted that there were few studies that had been adequately designed, conducted and reported that could be considered for purposes of classification of effects on the development. A majority of the Specialised Experts recommended no classification of cholecalciferol for effects upon the development. The arguments were that there is no evidence suggesting that the substance is teratogenic in humans, even at high doses. In animal studies, effects on development but not malformations occurred at dose levels, for which hypercalcemia had been demonstrated or must have been present. Other lesions in foetuses were considered non-specific and secondary to maternal toxicity. A large minority of Experts in favour of Category 3 argued that external, visceral and skeletal abnormalities observed in rabbit foetuses after administration of high doses of the active metabolite 1α,25-dihydroxyvitamin D3 (calcitriol) are specific and should lead to classification*" (citation from ECBI/ECBI/59/00 - Rev. 2).

The study performed with metabolite calcitriol (Rocaltrol®, Roche) in rats and rabbits is a publication and not a guideline study. It does not include all observations required by a guideline study and it is deficient with respect to reporting. Moreover, exposure was restricted to the period of organogenesis and only one third of foetuses were examined for visceral alterations.

Neverthelsess, the results showed no effects on development in rats up to a dose of 0.3 µg/kg bw/day. At this dose the serum calcium levels were increased and phosphate levels were decreased in both dams and offspring.

A more marked toxicity was observed in rabbits at 0.3 µg/kg bw/day, i.e. 18.8% mortality in the mothers and decreased 24 hour viability in the pups. Serum calcium was not measured in the rabbits. At the next lower dose i.e. 0.08 µg/kg bw/day, no mortality occurred in the mothers but 9/109 foetuses (in one litter) with microphthalmia and cleft palate (other effects also occurred, see table 3.8-2) were observed. This was not observed at 0.3 µg/kg bw/day but effects could have been masked by excessive maternal toxicity. At 0.3 µg/kg bw/day there were two foetuses with microphthalmia (in one litter) compared to one foetus in the control group. The study authors noted that there were no statistically significant differences between the treated groups and the control group with respect to the number of litters and foetuses showing abnormalities. They also noted the

uncertainty whether the multiple abnormalities observed in one litter each from the mid-and high-dose group were related to the test substance. This uncertainty is based on the low incidence of litters with effects, the lack of a clear dose response and the lack of statistical significance. Nevertheless, they could not discount a possible relation of effects with the test substance. The excessive maternal toxicity at the high dose of 0.3 µg/kg/day (death, weight loss, hypercalcemia with focal calcification of kidneys and lungs) co-occurred with foetotoxic effects (increase in resorption rate, decrease in average litter size and 24h survival index). Since maternal body weights, pathology and serum calcium data was not presented for the mid- and low dose groups, the impact of maternal toxicity cannot be fully evaluated. The dossier submitter agrees that the study has limitations but considering the frequency of developmental effects reported and that mortality was not observed at the middle dose, the study is considered relevant for classification.

There are other studies available in the open literature which are summarised in the synopsis in Doc IIIA.6.8.1-07. Since year 2000 the view with respect to the impact of maternal toxicity on classification for developmental effects has changed. Therefore, it is considered justified to re-assess these old studies although they were available at the time when the previous classification discussion on cholecalciferol took place. However, only those studies where vitamin D (ergocalciferol) was administered by a route relevant for classification have been evaluated further for developmental effects whereas others[2] have been excluded from further evaluation. The following studies were assessed in full by the dossier submitter: Zane 1976, Potvliege 1962, Nebel and Ornoy 1972, Ornoy 1971, Ornoy *et. al*. 1972a, Ornoy et. al. 1972. Moreover, two additional studies by the same authors Ornoy (Ornstein) *et. al*., 1968, Ornoy and Nebel 1969 have been assessed (Table 10.10.h).

In these studies, effects observed on rat foetuses include a decreased viability of foetuses after birth, decreased foetal weight, decreased bone mineral content, shortened bones and kyphoscoliosis in surviving foetuses with an increase of severity after birth. However these findings are not considered to fulfil criteria for classification since the effects were observed in the maternally lethal dose range and the studies are deficient with respect to methodology and reporting (e.g. the frequencies of specific effects were not reported).

The product literature of medicinal products[3] containing cholecalciferol (400 to 10000 I.U. per oral dose) informs that there are preclinical data indicating teratogenicity in test animals at high doses (i.e. doses greater than those used in clinical practice). The effects were described in one summary of product characteristics (SmPC) as malformations in rats, mice and rabbits (skeletal defects, microcephaly, and cardiac malformation) but as the full reference is not available in the summary of product characteristics for cholecalciferol the information cannot be scientifically assessed.

The vitamin D metabolism in pregnant rabbits has also been investigated in a published study (IIIA.6.2-03). A high dose of cholecaliferol of approximately 250 µg/day was given at 3 consecutive days before term. In this study, calcitriol (1α, 25-Dihydroxycholecalciferol, the

[2] These include studies using subcutaneous, intravenous or intramuscular administration as well as studies with effects on fertility at high doses where excessive toxicity was reported or can be expected, studies with administration to pups after birth, a study using an unkown dose, studies performed with in vitamin D deficient rats and two studies that were not available to the dossier submitter (i.e. Latorre 1961, Horii 1992, Mirzahosseini 1996, Friedman and Roberts 1966, Friedman and Mills 1969, Chan 1979, Zusman 1981, Ornoy 1981, Nebel and Ornstein 1966, Sharma 1972, Tshibangu 1975, Kistler 1980, Dostal 1983b,Yukikoka 1959, Jarnagin 1983, Kwiecinski 1989a, Kwiecinski 1989b, Uhland 1992, Makita 1977, Durr 1979).

[3] COMMISSION REGULATION (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin http://ec.europa.eu/health/files/eudralex/vol-5/reg_2010_37/reg_2010_37_en.pdf

metabolite regulating intracellular and extracellular calcium concentrations) did not increase following administration of cholecalciferol. This result is in line with a review suggesting that calcitriol does not increase with high doses of vitamin D (IIIA.6.2-02). In the study with pregnant rabbits, the intra-uterine foetal mortality was significantly higher in the cholecalciferol exposed foetuses compared to controls, i.e. 17.9% versus 2.8%. However, the decreased foetal viability should be interpreted with care since the study was limited with respect to methodology, reporting and investigations of toxicity. The only parameter reported for mothers and foetuses is viability and there is no information if the extent of hypercalcinosis was investigated. Nevertheless, the calcium levels were increased compared to controls at gestation day (GD) 29 in cholecalciferol treated animals. Although the increase was approximately 20%, it is questionable if it was the cause of the decreased foetal viability since the calcium level is yet within the range observed in non-treated females and were comparable to untreated pregnant females at GD 27-30. Overall, this study only provides supplementary information due to deficiencies in reporting and methodology.

High levels of calcitriol was also investigated in a recent study in pregnant female mice with a vitamin D receptor (VDR) deficient genotype (vdr-/-). The study, which is not performed according to any guideline, is considered insufficient for classification as stand-alone data as it was not designed to investigate effects on the standard developmental toxicity parameters at different doses. However, it is discussed here since serious effects of calcitriol directly affecting foetuses were reported. Calcitrol passed the placenta and affected foetuses by bone demineralisation and caused neonatal lethality. It was suggested by the author that the imbalanced calcium storage was the cause of the lethality in the pups. However in this study the mothers had high levels of calcitriol in the blood as a consequence of disrupted VDR signalling (due to the VDR null genotype). The VDR null mothers were also hypocalcemic. Foetuses were either VDR heterozygote (responsive) or VDR null (resistant) to calcitriol. In contrast to the results in this study, the pup survival was not affected in the rat teratogenicity study of calcitriol (IIIA.6.8.1-02) indicating the importance of the vitamin D Receptor. Calcitriol is a major metabolite of cholecalciferol and is hormonally active. Both substances cause hypercalcemia in test animals and humans which is generally considered the major toxicological effect. It can be discussed whether studies on calcitriol are relevant for hazard assessment of cholecalciferol since it has been suggested that calcitriol does not increase when overdosing with vitamin D (IIIA.6.2-02). On the other hand, calcitriol did increase in a clinical trial using doses up to 100 µg/day of cholecalciferol during pregnancy (IIIA 6.8.1/04). There were no effects on offspring observed in this study but it should be noted that there is no information from clinical trials on effects following repeated doses above 100µg/day during pregnancy.

Human data

The EFSA scientific opinion from 2012 refers to one systematic literature review performed by De-Regil *et. al*, in 2012 describing six randomized clinical trials (including 1023 pregnant women) on vitamin D alone or with calcium (vitamin D dose 20-30 µg/day). There were no difference in risk between vitamin D and non-vitamin D supplemented pregnancies with respect to the incidences of pre-eclampsia, nephritic syndrome, stillbirth or neonatal death. EFSA also found a study in lactating mothers where doses up to 100 µg/day did not affect offspring when given during 1-4 months of lactation. This study was a small trial. The EFSA panel reported that in this study there was no increase of serum calcium in the mothers and no hypercalciuria occurred. The serum 25-hydroxycholecalciferol concentration increased in the infants as a consequence of increased vitamin D intake via milk. EFSA also found a nested case-control study with a U shaped correlation between the metabolite 25-hydroxycholecalciferol concentrations before 22 weeks and the frequency of “small for gestational age” foetuses at birth. However, the EFSA panel noted that deficiencies such as lifestyle and dietary confounders were not fully taken into account and that no vitamin D intake data was available for linking to the observed effects. The overall conclusion by

EFSA on this study was to consider the study inconclusive for relating vitamin D intake to pregnancy outcome.

According to the report by the Nordic Nutrition Recommendations (the NNR5 project), two systematic literature reviews on pregnancy related outcomes were found that met their inclusion criteria. In one of these a nested case-control study was evaluated. In this study 274 healthy nulliparous women were followed from less than 16 weeks of pregnancy to delivery. The study suggested that low blood levels (<37.5 nmol/L) of Vitamin D may be associated with an increased incidence of pre-eclampsia. The second was the review by de-Regil *et. al.* that was also evaluated by EFSA and is described above.

The study reports for two additional clinical trials did not show any effects in newborns from mothers treated with up to 100µg/day of cholecalciferol or a single dose of up to 1.75 mg administered 10 weeks before term (IIIA 6.8.1/04 and IIIA6.8.1/05). The clinical trial with doses up to 100 µg/day was also included in the review by EFSA.

In a double-blind, randomised clinical trial of Vitamin D3 supplementation during pregnancy, 400, 2000 or 4000 IU/day (10, 50 or 100 µg/person) was given (IIIA 6.8.1/04). Dosing commenced around weeks 12 and 16 of pregnancy and continued throughout pregnancy. The calcitriol increase observed in this study was depending on the substrate availability of 25-hydroxycholecalciferol i.e. the major metabolite of cholecalciferol formed by the liver. There were no differences in safety outcomes for mothers and babies between the groups and no adverse events were attributed to cholecalciferol in any group. However, a limitation of the clinical trial was the study exposure being restricted to the period after early organogenesis.

A single oral dose of 70,000 IU (1750 µg/person corresponding to approximately 29 µg/kg bw in a 60 kg person) was given in another trial to 18 non-pregnant and 13 pregnant women in the third trimester (at least 10 weeks before term, IIIA 6.8.1/05). The primary objective of the trial was to measure pharmacokinetic parameters. Women were followed until one month post-partum. Supplementation did not induce hypercalcaemia, and there were no supplement-related adverse events.

EFSA found one small study from 1938 where retarded growth was observed in nine infants. The infants received various regimens of vitamin D exceeding 45 µg/day up to one year of age. Another small study using doses up to 54 µg vitamin D/day until about five months of age did not show such an effect. These inconsistent data were complemented with a recent Finnish population based cohort study that retrospectively assessed the association between vitamin D supplementation during infancy and body length or height at one year (measured), at 14 years age (self reported) and in adulthood (self reported and measured). The recommendations were 50 µg/day at the time of the study. There were no differences in height observed when groups were arranged according to frequency of supplementation (none, irregular or regular). However EFSA emphasizes that the groups were unequally sized.

In addition, recent intervention studies using doses up to 25 µg vitamin D/day (plus the amount ingested via fortified infant formula) for up to five months after birth did not indicate that intakes of up to 25 µg/day were associated with hypercalcaemia in infants (EFSA 2012 review).

In summary, human data can be found in different reviews but this information is not considered sufficiently robust to support an accurate assessment whether or not the intrinsic properties of the substance fulfils criteria for classification. The information available is scarce and confounding factors cannot be excluded. Moreover, information is restricted to effects of doses in the supplement range and to an exposure duration covering only a part of the gestation period.

10.10.6 Comparison with the CLP criteria

The data considered in the assessment of developmental toxicity include:

- Two non-GLP developmental studies with metabolite calcitriol in rats and rabbits.
- Two separate reports of clinical trials in humans.
- EFSA scientific opinion reporting data from studies of women administered vitamin D as a supplement during a part of pregnancy.
- A systematic Literature review within the NNR5 project including also data from studies of women administered vitamin D as a supplement during a part of pregnancy.

There are no standard OECD teratogenicity tests of cholecalciferol in animals available. Nevertheless, the effects observed in rabbits include cleft palate, microphthalmia, open eyelids and other skeletal abnormalities in 9/109 animals from a single litter at a dose without apparent severe toxicity to the mother. However due to deficiencies in reporting, it is not possible to scrutinize if there is any correlation between these effects in offspring and the toxicity in mothers. Therefore, it is not possible to conclude if effects could be considered non-specific and secondary to maternal toxicity. Microphthalmia was seen in 2/68 foetuses of the high dose group in which excessive toxicity also occurred (maternal death 19% and decreased foetal viability). The excessive toxicity may however have masked effects at the high dose. The frequency of microphthalmia was 1/195 in controls. The malformation was observed in one litter each in control, mid dose and high dose animals. There is no historical control data from the performing laboratory. However, although historical control data can be found in the open literature and although these types of malformations are rare in rabbits[4], the fact that they were restricted to the same litter indicates that they represent chance findings rather than an effect of treatment.

Some indications of intrauterine mortality was also observed in a cholecalciferol study investigating kinetics during pregnancy in rabbits but due to the limitations of the study, this data is not conclusive. Reduced foetal viability and bone effects, i.e. effects on bone mineral content, kyphoscoliosis and shortened bones have also been observed in rats when mothers were administered high doses in the lethal range.

Due to the limitations of the animal studies (i.e. deficiencies with respect to methodology and reporting) and the use of doses in the lethal or high toxicity range, the data is not considered sufficiently robust to accurately assess if the intrinsic properties of cholecalciferol fulfils criteria for classification. Therefore, classification for developmental toxicity is not proposed on basis of these studies which were also considered in the previous discussion on classification TC C&L (ECBI/76/00 - Rev. 3, 30.08.2001; ECBI/59/00 – Add. 1 - Rev. 2, 11.04.2001). The human clinical trials reported do not add any concern at supplemental levels.

[4] Discussed in the RAC opinion on Imidazole (http://echa.europa.eu/documents/10162/2f2a6450-be68-4238-9309-33053f63e14c)

10.10.7 Adverse effects on or via lactation

Table 10.10.j: Summary table of animal studies on effects on or via lactation

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
Not stated, Not GLP	The main deviations to OEDC 416 guideline were: No info on acclimatisation or previous treatments or age of animals. The number of pregnant animals was 20-24 but only 50% of these were allowed to deliver. The identity of the control solution was not given. Shorter pre mating period in males (60 vs 70 days). No assessment of sperm parameters. Females were treated over at least 2 oestrus cycles. No F2 generation, only F1 offspring studied until day 21 of lactation. There were no details on clinical observations given (nor if it was performed and how often). Body weights seem to have been recorded on a weekly basis (it is mentioned that statistics was performed on weekly body weights but results were not tabulated). Sex of pups and body weight of	Rat, Crl:CD 20/group	1α,25-Dihydroxyvitamin D3 (Calcitriol)	0, 0.02, 0.08, 0.3 µg/kg bw/day Gavage 60 days premating through pregnancy and lactation Perinatal and postnatal reproduction part of study: female rats were mated with untreated males and dosed from Day 15 of gestation through Day 21 of lactation.	**Parental:** **0.02 µg/kg/day:** ↓ Serum phosphate (-10%) **0.08 µg/kg bw:** ↓ Serum phosphate (-11%) **0.3 µg/kg bw:** ↓ Serum phosphate (-13%), ↑ Serum Calcium (11%) **Offspring:** **0.02 µg/kg/day:** ↑Urea nitrogen (25%) **0.08 µg/kg bw:** ↓ Serum phosphate (-6%) **0.3 µg/kg bw:** no stat .sign. changes in serum chemistry No stat sign. Changes in bone ash, No effect on % pregnant, average litter size, average no. of implantation sites/litter, % resorptions, average no. corpora	IIIA6.8.2-02

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
	pups was not given in the study, functional observations of pups was not made, terminal organ or body weights was not given, histopathology of reproductive or known target organs was not performed				lutea/litter, gestation-, viability-, lactation index	
Not stated, Not GLP	See above	Rat, Crl:CD 20/group	1α,25-Dihydroxyvitamin D3 (Calcitriol)	0, 0.02, 0.08, 0.3 µg/kg bw/day Gavage Perinatal and postnatal reproduction part of study: female rats were mated with untreated males and dosed from Day 15 of gestation through Day 21 of lactation. Serum chemistry in 20 dams/group and serum chemistry and bone ash measurements in 2 pups/sex and litter	**Parental:** **0.02 µg/kg/day:** No stat sign changes **0.08 µg/kg bw:** ↓ Serum phosphate (-15%) ↑ Serum calcium (20%) **0.3 µg/kg bw: ↓** ↓ Serum phosphate (-15%) ↑ Serum Calcium (29%) ↑Urea nitrogen (26%) **Offspring:** **0.02 µg/kg/day:** No stat sign changes **0.08 µg/kg bw:** ↑ Serum calcium (20%) **0.3 µg/kg bw: ↑** Serum calcium (23%)	IIIA6.8.2-02

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
					Changes in bone ash was not stat. sign. although an increase occurred in treated groups	
Not stated, Not GLP	No guideline was followed. The following investigations was reported: Serum calcium was reported as mean ±SE from 8 mothers and one pup from 8 litters at day 12 and 17 (dietary exposure) Serum calcium was reported as mean ±SE from 8 mothers and 8 pools of two pups/litters on day 8, 14-16 individual pups day 11 Vitamin D metabolite profile was investigated in milk an plasma from rats injected ip daily dosing for 5 days from lactation day 4 with 0.18 or 0.29 µCi [^{3}H] cholecaliferol plus 1 µg/g bw or cholecaliferol 0.18 or 0.29 µCi [^{3}H] cholecaliferol alone (controls). The lower dose was first given and analysed before the	Rat (strain not identified in the publication) .	Chrystalline cholecalciferol (sigma St. Louis, MO). Purity was not given.	300IU cholecalciferol/g diet GD 20 or lactation day 1 1µg cholecalciferol /g bw orally from day 3 of lactation Duration 8 or 12 days Before treatment the animals were maintained on diet with 5 IU/g diet of cholecalciferol (control vitamin D diet for dietary high dose and controls) For orally dosing of high doses the controls were given this diet and the olive oil vehicle.	<u>Mothers</u>: a) High dose in diet: Increased serum calcium day 12 and 17 of lactation 1-2 mg/dl, P<0.01), no difference in serum P or kidney of heart Ca content (reported but data not shown) b) High dose orally: Increased serum Ca day 6 and 11 of lactation (atleast 1 mg/dl, P<0.01) Serum P increased by 1 mg/dl Kidney Ca content + 20%, P<0.01, heart Ca content unafeccted. <u>Offspring (both treatments):</u> Increased serum Ca 1.5 mg/dl (p< 0.025 and P >0.01), P not affected, 2x increase kidney Ca in one high	Dostal et. al 1983

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
	remaining three were given their dose. The remaining three was given a higher radioactivity (recovery and distribution of radioactivity did not differ between the doses and the data was combined). Blood and milk was sampled 24h after the last dose Milk was analysed day 10 of lactation for % milk solids, % ash, Ca (mg/g solids), Mg (mg/g solids), P (mg/g solids). N= 6 controls, 5 given 1µg/g bw cholecalciferol day 3 of lactation. Serum phosphorous, body weight and kidney calcium content was analysed in pups, heart calcium and kidney calcium was analysed in mothers but the results was only briefly presented This study also investigated effects of daily oral doses of 2 ng/g bw of calcitriol treatment in lactating rats. Statistical analysis students one tailed				diet experiment (no data details presented), body weight unaffected (data not presented) High dose calcitriol to lactating rats kept on diet with 5 IU/g cholecalciferol also gave increased serum Ca in mothers but not in pups and the milk Ca, Mg or P did not change in animals kept on a vitamin D deficient diet. <u>Milk:</u> Controls/High oral dose cholecalciferol: serum Ca: 9.55/11.03 (P<0.01) % milk solids: 23.2/24.4 % Ash: 4.63/4.43 Ca (mg/g solids): 9.89/9.54 Mg (mg/g solids): 0.78/0.78 P (mg/g solids): 6.84/6.62 <u>Vitamin D metabolite content of plasma and milk:</u> Majority % of total cpm comigrating with standard was cholecalciferol 48.2- 84.6% in	

Method Guideline	Deviation(s) from the guideline (if any)	Species Strain Sex no/group	Test substance, reference to table 5	Dose levels duration of exposure	Results	Reference
	t-test				control and treated sera mothers and pups, milk Followed by 25OHD range 6.4-34.6% 24, 25 $(OH)_2D_3$ up to 8.3% and calcitriol only detected in control mothers plasma. Recovery range 42.5 to 72.2% High oral calcitriol dosing	

Table 10.10.k: Summary table of human data on effects on or via lactation

Type of data/report	Test substance, reference to table 5	Relevant information about the study (as applicable)	Observations	Reference
EFSA Sci. opinion				See section 10.9.b
Systematic Literature Review (NNR5 project)				See section 10.9.b

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

High oral doses of cholecalciferol in diet (300 IU/g diet) from gestation day 20 or day 1 of lactation or daily oral dosing of 1 µg/g bw from lactation day 3 for 1 or 8 days resulted in significant increases in serum calcium in mothers and in rat pups. The content of calcium, magnesium and phosphor in milk was unaffected by treatment but the milk contained high levels of cholecalciferol (Dostal et. al. 1983). Investigations of toxicity in the pups were limited to analyses of serum calcium, body weight and kidney calcium content. The effects were reported as the mean values for eight rats (high levels in diet) and eight mothers. The body weight of pups were claimed to be

unaffected by treatment but there is no data to support this statement in the publication. The serum calcium levels increased by 1.5 mg/dl in pups and 1-2 mg/dl in the mothers and a twofold kidney calcium content increase occurred. Due to limited reporting of effects in pups, it is not known whether these increased levels in milk were associated with any toxic effects.

However, intake of vitamin D is considered essential for normal growth and development. Deficiency can cause rickets in children hence supplements are given to children. The EFSA scientific opinion reports results from one study in 18 lactating women with limited sun exposure who randomly received either 40 µg vitamin D2+10 µg vitamin D3/day or 90 µg vitamin D2+10 µg vitamin D3/day from month 1 through 4 of lactation. Serum calcium concentrations remained within the normal range and hypercalciuria did not occur. The serum concentration of metabolite 25-hydroxycholecalciferol increased in infants following treatment of the mothers but there were no adverse effects reported in the infants.

The NNR5 systematic literature review reported six trials of vitamin D supplementation in pregnant and lactating women. All of these are considered to be of low quality with respect to methodology. Nevertheless, according to the systematic literature review, the studies showed that a dose of 25-90 µg/day increased the maternal serum level and the cord blood level of metabolite 25-hydroxycholecalciferol. They also found that in one of the trials, administration of 25 µg/day to lactating mothers did not increase the serum concentration of 25-hydroxycholecalciferol in the infant. Furthermore, the review identified many studies suggesting an increased risk of rickets in infants and children at low serum levels of 25-hydroxycholecalciferol but they did not find sufficiently robust data to be able to conclude on the optimal serum level (or intake level) in children, adolescents or adults.

10.10.9 Comparison with the CLP criteria

Regulation (EC) 1278/2008 states the following: "*Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk*".

The data available to assess this hazard class include human data discussed in the systematic literature review within the NNR5 programme and the rat study by Doastal *et al* 1983.

This data contain no information whether or not exposure via lactation can result in adverse effects in the infant thus there is no "human evidence indicating a hazard to babies during the lactation period" and consequently, criterion (a) is not fulfilled.

Based on the limited study in rats performed during the peri/postnatal period, there is some evidence that the active metabolite calcitriol causes hypercalcemia in the pups during lactation but there is no information whether or not this was accompanied by any toxic effects in the pups. Therefore, criterion (b) is not fulfilled.

However, the data do give some support for criterion (c) since the rat study shows transfer of cholecalciferol into the milk of lactating female rats resulting in elevated serum calcium levels in the suckling pups. Due to the limited reporting, the level of toxicity in pups is not known. Cholecalciferol is essential for infant growth but effects following exposure to doses above the range used for supplement cannot be excluded.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

Due to limitations in the data on reproductive toxicity, i.e. lack of thorough investigations of all parameters required for an accurate assessment of effects on fertility and developmental toxicity and/or deficiencies with respect to methodology and reporting, data is not considered sufficient to assess if the intrinsic properties of cholecalciferol fulfils criteria for classification. Therefore, no classification with respect to fertility, teratogenicity or lactation is proposed.

10.11 Specific target organ toxicity-single exposure

According to the CLP Regulation and the Guidance on the Application of the CLP Criteria (Version 4.0 – November 2013), STOT-SE Category 1 and 2 is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature with significant impact on health. Both factors have to be evaluated by weight of evidence and expert judgement.

The deaths observed in the acute toxicity studies occur within the same range as the guidance values for STOT SE 1, i.e. C ≤ 300 mg/kg bw (oral, rat), C ≤ 1000 mg/kg bw (dermal, rat or rabbit) and C ≤ 1.0 mg/l/4h (inhalation, rat). In acute oral studies performed with Sprague-Dawley rats and Wistar rats there were clinical signs of neurological nature observed also at doses slightly below those causing deaths. The clinical signs observed include hypoactivity and ataxia in female Sprague-Dawley rats at 146 mg/kg bw and signs such as apathy, decreased alertness and startle response, decreased locomotor activity, abnormal body posture and gait, piloerection, passiveness, decreased body tone, ptosis, paralysis, loss of grooming, respiration difficulties in Wistar rats at 12.5 mg//kg bw/day (see section 10.1). However, since mortality occurred in some of the animals at the next dose levels in both studies (i.e. 219 mg/kg bw in Sprague-Dawley rats and 25 mg/kg bw in Wistar rats), these symptoms likely reflect general discomfort rather than a specific effect on the nervous system. Also in the dermal study there were clinical neurological signs occurring at the same doses that produced lethality. Likewise, clinical signs indicative of an effect on the mood, motor activity and coordination, posture, muscle tone and the autonomic nervous system were observed in the inhalation study but these signs occurred at lethal doses. No signs of respiratory irritation were observed during exposure (estimated as clinical signs and effect on respiratory frequency, nor were there any dose related increase in lymphocytes in lung tissue). Macro- and microscopic observations revealed calcification of the lungs and visceral organs and the lung weights (relative and absolute) were increased in females. Body weights were decreased during follow up period but regained control levels.

In summary, the clinical signs could be caused either by hypercalcemia, acute neurotoxicity or be indicative of a more generalized toxic response. Since no biochemical analyses were made in the acute oral toxicity studies, it is not possible to determine if the neurological effects observed were secondary to hypercalcemia. However, pathology of animals that died during the observation period or were killed after the observation period showed hypercalcinosis of heart, spleen, kidney and blood vessels at a dose of 25 mg/kg bw and above. Since the effects occurs within the same dose

range as the acute oral toxicity, the clinical signs are considered to be caused by generalised toxicity rather than a specific target organ toxicity and are thus covered by the classification for acute toxicity.

Currently, the criteria for classification in STOT SE Category 3 only cover the transient effects of 'respiratory tract irritation' and 'narcotic effects'. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. Substances are classified specifically for these effects when meeting the criteria laid down in section 3.8.2.2 on CLP. Section 3.8.2.2.1 of CLP lists the criteria for respiratory tract irritation. No signs of respiratory irritation (estimated as clinical signs and effect on respiratory frequency), no dose related increase in lymphocytes was determined in lung tissue and no narcotic effects were observed during exposure to cholecalciferol.

Therefore, cholecalciferol is not considered to meet criteria for classification STOT SE.

10.12 Specific target organ toxicity-repeated exposure

As described in the previous section, STOT Category 1 and 2 is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature with significant impact on health.

Cholecalciferol is currently classified STOT-RE**, which is a classification translated from the previous legislation, i.e. according to Directive 67/548/EEC. The CLP guidance value for classification as STOT RE in category 1 after a 90-day repeated-dose study is C ≤ 10 mg/kg bw/day (oral, rat). In the studies available for this assessment, effects observed and considered relevant for this category include progressive hypercalcemia with tissue mineralisation in several organs and proliferative adrenal pathology since these were observed already at doses of 0.06 mg/kg bw/day and 0.3 mg/kg bw/day, respectively, in the 90-day rat study (Table 10.9.d). The effects are consistent between studies and indicate an impaired organ function at doses that are well within the guidance value set in the CLP regulation for a 90 day study in rats. The existing classification as STOT RE 1 is therefore confirmed.

Tissue mineralisation was observed in many organs examined in the 90 day study (i.e. aorta, heart, kidneys, stomach, trachea, femur and sternum (hyperostosis). Although associated microscopic findings were only seen in the kidneys (degeneration/regeneration, tubular dilation and proteinaceous cast), the dossier submitter hesitates to pinpoint the kidneys as the target organ taking into consideration the possible influence of study conditions and that further effects could have been noted if data would have been available for several species. Moreover, generalised tissue mineralisation seems consistent with the mode of action of the substance, i.e. mobilization of calcium from the bone matrix to plasma and death from hypercalcemia.

There is no repeated dose toxicity data available for the dermal or inhalation route. The Guidance on the Application of the CLP Criteria (Version 4.0) informs that for this situation, with only data available for one route, no route should be stated.

Based on the equations in the Guidance on the Application of the CLP Criteria, the specific concentration limits assigned to the substance and applicable to mixtures containing the substance would be the following:

Equation 3.9.2.6.a SCL*Cat*.1=(ED/GV1) x 100% or SCL*Cat*.1= (0.06/10) x 100= 0.6%

Equation 3.9.2.6.b SCL*Cat*.2=(ED/GV2) x 100% or SCL*Cat*.2= (0.06/100) x 100= 0.06%

10.13 Aspiration hazard

Hazard classes not assessed in this dossier

11. EVALUATION OF ENVIRONMENTAL HAZARDS

Hazard classes not assessed in this dossier

12. EVALUATION OF ADDITIONAL HAZARDS

Hazard classes not assessed in this dossier

13. ADDITIONAL LABELLING

Not relevant

14. DETAILED STUDY SUMMARIES

The study summaries of the draft CAR (Doc IIIA) containing data relevant to the hazard classes assessed in this dossier are included in a confidential annex to the technical dossier (IUCLID).

14.1 PHYSICAL HAZARDS

Hazard classes not assessed in this dossier

14.2 TOXICOKINETICS

See separate annex with CAR study summaries

14.3 HEALTH HAZARDS

14.3.1 Acute oral toxicity

See separate annex with CAR study summaries

14.3.2 Acute dermal toxicity

See separate annex with CAR study summaries

14.3.3 Acute inhalation toxicity

See separate annex with CAR study summaries

14.3.4 Skin corrosion/irritation

Hazard class not assessed in this dossier

14.3.5 Eye damage/eye irritation

Hazard class not assessed in this dossier

14.3.6 Respiratory sensitisation

Hazard class not assessed in this dossier

14.3.7 Skin sensitisation

Hazard class not assessed in this dossier

14.3.8 Germ cell mutagenicity

See separate annex with draft CAR study summaries relevant for this hazard class

14.3.9 Carcinogenicity

See separate annex with draft CAR study summaries relevant for this hazard class

14.3.10 Reproductive toxicity

See separate annex with draft CAR study summaries relevant for this hazard class

14.3.11 Specific target organ toxicity

See separate annex with CAR study summaries

14.3.12 Specific target organ toxicity (repeated exposure)

See separate annex with CAR study summaries

14.3.13 ASPIRATION HAZARD

Hazard class not assessed in this dossier

14.4 ENVIRONMENTAL HAZARDS

Hazard class not assessed in this dossier

14.5 ADDITIONAL HAZARDS

Hazard class not assessed in this dossier

15. REFERENCES

A list of references for the study summaries of the draft CAR (Doc IIIA) is included in the confidential Annex 1 which is attached to the technical dossier (IUCLID).

1. Bayer CropScience. Position Paper: Cholecalciferol - Comet test in the rat: systemic toxicity and assessment of glycogen depletion in the liver. Unpublished, November 26th, 2014.

2. Blomberg Jensen M. Vitamin D metabolism, sex hormones, and male reproductive function. Reproduction. 2012 Aug;144(2):135-52.

3. Dostal LA, Boass A, Toverud SU. Effects of high doses of vitamin D3 and 1,25-dihydroxyvitamin D3 in lactating rats on milk composition and calcium homeostasis of the suckling pups. Endocrinology. 1983 May;112(5):1631-8.

4. EFSA Scientific Opinion on the Tolerable Upper Intake Level of vitamin D. EFSA Journal 2012;10(7):2813, http://www.efsa.europa.eu/en/efsajournal/pub/2813.htm

5. Greim H, Hartwig A, Reuter U, Richter-Reichhelm HB, Thielmann HW. Chemically induced phaeochromocytomas in rats: mechanisms and relevance for human risk assessment. Crit Rev Toxicol. 2009;39(8):695-718.

6. Isobe K, Ito T, Komatsu S, Asanuma K, Fujii E, Kato C, Adachi K, Kato A, Sugimoto T, Suzuki M. Stimulation of adrenal chromaffin cell proliferation by hypercalcemia induced by intravenous infusion of calcium gluconate in rats. J Toxicol Pathol. 2012 Dec;25(4):281-5.

7. Lerchbaum E, Obermayer-Pietsch B. Vitamin D and fertility: a systematic review. Eur J Endocrinol. 2012 May;166(5):765-78.

8. Nebel L, Ornoy A. Interdependence of fetal anomalies and placental impairment following maternal hypervitaminosis D and hypercortisonism. Adv Exp Med Biol. 1972;27:251-5.

9. Ornoy A, Horowitz A, Kaspi T, Michaeli Y, Nebel L. Anomalous fetal and neonatal bone development induced by administration of cortisone and vitamin D2 to pregnant rats. Adv Exp Med Biol. 1972;27:219-26.

10. Ornoy A, Kaspi T, Nebel L.Persistent defects of bone formation in young rats following maternal hypervitaminosis D 2 . Isr J Med Sci. 1972 Jul;8(7):943-9.

11. Ornoy A, Nebel L, Menczel Y. Impaired osteogenesis of fetal long bones. Induced by maternal hypervitaminosis D2. Arch Pathol. 1969 Jun;87(6):563-71.

12. Ornoy A, Menczel J, Nebel L. Alterations in the mineral composition and metabolism of rat fetuses and their placentas induced by maternal hypervitaminosis D2. Isr J Med Sci. 1968 Jul-Aug;4(4):827-32.

13. Ornoy A The effects of maternal hypercortisonism and hypervitaminosis D2 on fetal osteogenesis and ossification in rats. Teratology. 1971 Nov;4(4):383-94.

14. Potvliege PR. Arch Pathol. 1962 May;73:371-82. Hypervitaminosis D2 in gravid rats. Study of its influence on fetal parathyroid glands and a report of hitherto undescribed placental alterations.

15. Tischler AS, Powers JF, Downing JC, Riseberg JC, Shahsavari M, Ziar J, McClain RM. Vitamin D3, lactose, and xylitol stimulate chromaffin cell proliferation in the rat adrenal medulla. Toxicol Appl Pharmacol. 1996 Sep;140(1):115-23.

16. Zane CE.Assessment of Hypervitaminosis D during the first trimester of pregnancy on the mouse embryo. Preliminary report. Arzneimittelforschung. 1976;26(8):1589-90.

16. ANNEXES

The study summaries (doc IIIA references) of the draft CAR are included in the confidential attachment (annex I) to the technical dossier (IUCLID).